

Electronics in trouble worldwide

Two years of upheaval in the market for microprocessor computers have now been followed, in Britain, by stockholders' uneasiness about electronics giants. Stoicism is called for.

WHAT has gone wrong with the electronics industry? For the past decade, as the benefits of integrated semiconductor devices have become apparent not merely in the design of novel equipment such as personal computers but also in telecommunications equipment serving a traditional purpose in a novel way, prophets have been singing the praises of what the future will be like, and those with money to invest have believed what they have heard. The recent past has been traumatic for them. In the United States, the troubles began more than two years ago, when mushroom companies, large as well as small, found that their financial accounts were having to be written mostly in red. Many have since gone out of business, others have been bought up cheaply by their competitors. It is not surprising that the New York stock markets now take a more cautious view of the attractions of investing in electronics companies. Nor is it surprising that the rot has now spread outside the United States, to Britain in particular. Some manufacturers of personal computers (such as the Wales-based Dragon) went to the wall almost as soon as they began. More recently apparently solid companies have run into trouble. Acorn, the benefits of an arrangement with the BBC notwithstanding, has had to be rescued by Olivetti but may yet fail. Sinclair has been bought up by an offshoot of Mr Robert Maxwell's Pergamon Press. Now, to the general consternation, some of the giants of British electronics manufacturing are deeply offending their owners. Stockholders are asking whether the future has been indefinitely postponed.

Rail fever

Small comfort though it may be, the present trouble in electronics is not without precedent. British economic history includes one of the most vivid illustrations of how the rapid development of a new technology may be a mixed blessing for those tempted to stake their fortunes on it—the development of the railway system. During the period from 1840 to 1870, George Stephenson's example served as a powerful stimulus of investment in new railway companies. In the event, many were geographically too confined to be viable except as adjuncts of larger companies, others were discovered to have grossly overestimated the markets for which they were established and went out of business, still others (as the railway map of Britain in the 1950s shows clearly) duplicated what other companies were about. By the end of the nineteenth century, the Victorian railway boom had enabled some of those who backed it to make great fortunes, but had robbed many others of their shirts. The recent upheaval among manufacturers of personal computers has clearly run along very similar lines, but the time-scale of events has been dramatically foreshortened.

In retrospect, the course of events should nevertheless be no surprise. Companies such as Atari grew quickly on the back of a fashion of the late 1970s, young people's interest in playing

games of a novel kind on television screens. But soon after Atari had been bought by Warner Communications, the gingerbread began to go stale as the fashion changed. Warner itself seemed for a time financially vulnerable. Among manufacturers of more seriously intended personal computers, there have been two recurrent technical problems, a persistent tendency to underestimate the length of time needed to turn a good idea into a workable (and saleable) device (software as well as hardware) and the maddening issue of the degree to which one machine is compatible with another.

Service

The persistent misjudgement of market needs, especially by the smaller manufacturers, is however much more relevant. In a field so novel, for example, the need for after-sales service to help customers use what they had bought should have been plain. Yet many companies, some of them casualties of the upheaval of the past two years, appear to have left that need out of account. But is not providing such a service, especially internationally, bound to be both intricate and expensive? That is what the smaller manufacturers have been saying. Of course. But what purpose is served in the long run by marketing even technically excellent products without providing what must be regarded as services essential to their proper use? In the unsophisticated markets of a few years ago, many smaller companies calculated that buoyant sales of low-priced hardware would give them a niche from which they might afterwards expand. One of the outstanding characteristics of this part of the computer market is that the cost of hardware may be only a fraction of the total, at least when some account is taken of the time unsophisticated users must spend on learning to use what they have bought. This truth, an awareness of which is now spreading, no doubt accounts for the recent slump in sales of even technically excellent machines. Investors will no doubt take note.

The more surprising recent faltering of confidence in major British companies must be differently explained. Part of the trouble is worldwide. In New York, even IBM's share price has not continued its buoyant upward surge as people have begun to realize what should have been evident, that the years immediately ahead are bound to see both high investment in research and development and fierce competition in the marketplace. The acquisition of the communications company MCI by IBM two weeks ago will, for example, bring about novel competition with established providers of telecommunications services, in which the profits of all concerned may suffer, to the general benefit of the users of these services. That, at least, is what should happen in the United States and possibly elsewhere if the regulators and legislators who have recently been deregulating the communications market do not lose heart.

The British scene is, as always, muddier, and should be an object-lesson both for governments and investors. One powerful influence has been the conversion of British Telecom from a public communications monopoly to a private near-monopoly. The immediate result has been to destroy the market for all except the most up-to-date capital equipment in the British network. Suppliers of analogue exchange equipment, on the development of which British Telecom's predecessors and the major British suppliers were engaged as recently as 15 years ago,

Biological manuscripts

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are now having to close manufacturing plant (which they might have expected), while the purchaser under whose umbrella they used to shelter now plans to play the international market for digital exchange equipment. The moral is that private companies that become over-dependent on a single customer (in Britain, Plessey and STC seem to be worst hit) are vulnerable in two respects: technically their pattern of activity is constrained, financially they depend on the whim of others.

There are also more immediate problems. The general depression and competitiveness of the computer market has made STC's purchase of the British company ICL seem less of a bargain than it did last year. Fierce international competition for general-purpose semiconductor chips, which has meant that prices have fallen by a factor of five within a year, has similarly afflicted the British company Thorn-EMI, the purchaser last year from the British government of Inmos, the semiconductor company. Those with an interest in these questions are now wondering which chairmen will be forced to leave which companies. Those with longer memories may recall the curious and still unexplained outburst last month by Lord Lucas, a government spokesman in the House of Lords, who surprised his audience with a recipe for the amalgamation of British electronics companies. The most obvious candidates for shotgun marriages are the companies whose balance-sheets (and share prices) have been hit by increased competitiveness of the general electronics market, but even companies such as GEC (no relation of US General Electric), which have stuck to customized semiconductor electronics for the most part, have disappointed their shareholders while maintaining their profits.

The outcome of this period of upheaval cannot, of necessity, be predicted. Indeed, even companies that have been hard hit in the past few weeks are strong industrial organizations, well able to ride out the storm. But the best-provided must be those whose interests span more than electronics pure and simple. They are best placed to swallow one or more of the others. The snag, for disappointed investors, is that in the present climate they may not wish to do so.

If the immediate future cannot be foretold, the more distant future is fortunately more predictable. Electronic computers with hugely different capacities are now well-established and productively used, while there is likely to be a lull of several years before machines of radically different architecture are working tools. So too are the technologies of telecommunications. But the marriage of the two fields has so far been half-hearted. That is why, with the increased competition between the giants of the two industries, the next decade should throw up novel opportunities for the smaller fry to become suppliers of specialized equipment; there should be plenty of niches for other companies to fill. Investors should know, however, that capital investment in research and development will be more than ever needed if they are to be filled. Meanwhile, ambitions to overtake the giants by sheer cleverness (which is not in short supply) should be surpassed. □

OPEC in the doldrums

OPEC is going through a bad patch, but remains a potentially powerful cartel.

OPEC, the Organization of Petroleum Exporting Countries, seems these days to stagger from one crisis to another. Last weekend's meeting in Vienna was no exception. After three days of talk, OPEC failed to persuade itself that there should be a system of production quotas that would restrict the amounts of oil that could be sold on world markets. Even the proposal that the total volume of OPEC production should be reduced by 7 per cent, unenforceable without production quotas, is only a formality, setting a ceiling (of 14.9 million barrels a day) believed to be higher than OPEC members now sell collectively. It is always possible that the economic climate may change before the next formal meeting of the organization, scheduled to begin on 22 July, but the prospects (for OPEC members at least) are

not bright.

Why is OPEC now apparently so weak when it seemed, in 1973 and 1979, that it could fix whatever price it chose for a barrel of crude oil and still keep its members' oil revenues at levels that satisfied their needs for overseas currency? The time-lag between 1979 and now is significant and the explanation is almost banal. The sudden jumps of price in the 1970s were shocks to the economies of the oil consumers. The short-term effects included all kinds of economic problems, stagflation in particular. One of the long-term consequences has been simple old-fashioned price resistance. Customers reconciled to prices of between \$10 and \$12 a barrel (the official price of Saudi Arabian crude oil between the first and second shocks) have changed their ways, and have taken steps to use less oil. In retrospect, it is unlikely that OPEC would have dared put the price above \$30 a barrel in 1979 if it were not that Iranian production had been halted. Since then, however, the trend of prices for the oil products sold on the Rotterdam market has been steadily downwards, in dollars and without allowing for inflation.

The driving force behind these dramatic changes is the way in which the oil consumers have managed to economize in its use. It is almost as if OPEC's 1979 round of price increases had been designed to bear out the contention of the late Shah of Iran that higher prices were the best way of ensuring that oil would be left in the ground for "future generations" to use as a source of chemicals.

The industrial trends are neatly illustrated in British Petroleum's latest *Statistical Review of World Energy*, published last month. Between 1970 and 1984, the consumption of primary energy in the United States for each unit of gross domestic product (GDP) fell by some 17 per cent. In the other industrialized countries which are members of the Organization for Economic Cooperation and Development, where energy consumption per unit of GDP has always been roughly half that in the United States, the fall since 1970 has been more dramatic — more like 20 per cent than 17 per cent.

Two quite separate trends are apparent in the figures. First, all of the industrialized countries are using less energy for each dollar's worth of wealth that they generate internally. Second, within the total pattern of energy consumption, the importance of oil is steadily declining. All this is what would have been predicted from the elementary rules of supply and demand. The only surprise is that there has been such a noticeable change in a mere six years.

So does this mean that OPEC is now finished as a determinant of the price of oil and, by extension, of other forms of energy? That is not a reasonable calculation. Part of the reason why oil consumption has been depressed since the beginning of the decade is that economic activity has been at a low ebb, and part of the reason for that long depression was the sharp increase of the price of oil. OPEC's strength has during that period been contained by a kind of negative feedback which few industrialized economies have enjoyed. Ominously, with the improvement of economic activity between 1983 and 1984, there seems to have been a sharp increase of total energy consumption which restored energy consumption to the peak that it reached in 1979. (GDP had increased in the interval.) There is always a possibility that the full restoration of prosperity will have oil companies bumping up against the now much reduced level of production by the OPEC states, for oil is for technical reasons the most ready way of meeting marginal demands.

The moral in this for the oil consumers is that they must hasten plans for the use of other energy sources. Coal consumption has grown steadily, but only slowly, in the past decade, and there is plainly a limit to the speed with which supplies can be increased. Nuclear energy similarly lives on a long time-scale (that could and should be shortened). Energy conservation measures are similarly slow to take effect. But these and other devices are, if anything, more necessary now than at any time in the past decade. The unpalatable alternative is to see the price of oil increase yet again. □

Japanese science

Selected council to replace over-democratic body

Tokyo

THE newly selected Science Council of Japan is to go into action later this month. And selected, rather than elected, is the word, for the government has had its way and turned the often-troublesome freely-elected "parliament of scientists" into a select advisory body. In the process, nearly 80 per cent of its members have been replaced.

Since 1949, the Science Council of Japan has been advising the government on science policy and promoting and coordinating scientific research both nationally and internationally. Its activities have been directed by its 210-member general council and performed through standing committees and research liaison committees involving some 1,500 scientists.

Council members used to be elected in a postal ballot, held every three years, in which virtually all of Japan's 240,000 scientists were qualified to vote. The council was thus perhaps unique among nations in providing all scientists with the right to participate directly in choosing representatives to the highest level of government: the chairman of the council had an automatic right to membership of the Prime Minister's Council for Science and Technology. Numerous successes were scored by the council over the years and many of Japan's finest research facilities grew out of its recommendations including the Nobeyama radio telescope, the Photon Factory, the Institute of Molecular Science and the Polar Research Institute.

But the council ultimately fell prey to a peril for all "grass roots" democracies, the apathy of the "moderate majority". The council has always contained a "peace" lobby which irritated the government by its opposition to nuclear power, by action that successfully prohibited research on nuclear weapons, and by demanding parliamentary action to guarantee "freedom of research" rights of scientists. It was such people that campaigned most vigorously at election time, not a cheap process for although only the sending of promotional postcards was permitted, many candidates spent as much as a million yen (US\$4,000). Over the years they gained a larger voice in the council.

In return, the government tended to pay less attention to council advice and relied more on its own advisory bodies such as the Ministry of Education's own science council. And that meant that many voters, perceiving the council's power to have lessened, became indifferent to its activities and abandoned it to "left-wingers" whose interest was more

political than scientific, or that at least is the story the government would have people believe. The affairs of the council were costing a lot of money, which had to be found by the government. Supervising elections alone, with an electorate of nearly a quarter of a million, cost 100 million yen (US \$400,000 dollars).

After a few years of dispute with the government (see *Nature* 305, 361; 1983), the council is now completely reformed (or rendered subservient, depending upon the point of view). Representatives are not directly elected but are chosen at meetings of an electoral college (the Council Selection Management Committee) which is elected, together with candidates for council membership, by the major academic societies.

French science

Horizon freed from bonds

Paris

FRENCH science seems set for another good year and perhaps for three. When the budget of the French ministry of research came up for parliamentary approval at the end of June, the Prime Minister himself, M. Laurent Fabius, opened the debate.

He told the assembly that "when the question is research, the future of our country is at stake", and said that "the whole effort of this government is found on the triangle of research, training and investment". The fact that the opposition walked out is taken at the ministry as a positive sign for 1986, when the opposition seems likely, by all opinion polls, to be returned to power. Abstentions, or a vote against the budget targets, would have been much worse than a walk-out.

The divided opposition in France, led by M. Giscard d'Estaing, M. Jacques Chirac and M. Raymond Barre, can hardly bring itself to vote for anything that the present government has done, when the popularity of the President, M. Francois Mitterrand, is at a low ebb. But science and technology are widely thought to be among the government's successes. Thus the innovation director of a Paris company, who "cannot stand" the present government, admitted last week that the attitudes of French academics have now "completely changed". Previously, he said, academics liked to prove that they had no time for practical affairs. Now, they compete for industrial clients.

As for money, M. Fabius told the national assembly, "two periods have marked the development of science in

The new system, along with a rule preventing anyone serving more than three terms of three years each, has led to a dramatic shift in council membership (plus a huge reduction in expenses). Of the 210 members, 163 are new. In come many of the most famous names in Japanese science, as well as a large number of very active researchers who had earlier refused to be involved. The average age of council members rises a couple of years to nearly 63, perhaps because of the influx of emeritus professors, and so does the number from the Tokyo region, up to 64 per cent of the total. Tokyo University professors alone take 17 per cent of the places, with Kyoto next at 8 per cent. Women too have done a little better, if a change from just one representative to a mere three can be called an improvement.

There is no doubt that the new council contains an extremely distinguished group of scientists who will put science before politics. The test will be whether they pay attention to the wider implications of the expansion of science they will no doubt try to promote.

Alun Anderson

France. One was under President Mendès-France and one under General de Gaulle." There was a great decline after de Gaulle (beginning with President Pompidou) in which French research and development spending fell from 3 per cent of the gross national product to 1.7 per cent. Now it has been pulled back to 2.25 per cent, which marks a very strong growth since 1980. However, Fabius was careful in his choice of date: M. Mitterrand and his government came to power in 1981, but the science budget was already beginning to increase substantially in 1980 under President Giscard d'Estaing's science minister M. Pierre Aigrain. So science spending is not a party issue.

In the meantime, M. Fabius promised to give science "an absolute priority", backed M. Curien's proposal to raise the research budget by 4 per cent a year in real terms to 1988, and set his country's sights on reaching 3 per cent of gross national product by 1990-91. "No science policy is possible without continuity", Fabius said. M. Curien's "three year plan" will create 1,400 new scientific posts per year, will increase the number of student grants and "develop mobility".

Other themes raised by M. Fabius were the linkage of the research community to industry; a "primordial" commitment to scientific development of Europe as a whole and promotion on merit. In forging links with industry and in returning to an academic meritocracy, the present government may ironically have had its greatest success — and lost most of its ideological socialist support.

Robert Walgate

West German universities

Erosion of plans for change

Hamburg

NEARLY 40,000 students took part in a demonstration against the proposed amendments to German university regulations in Bonn during June, marking another crisis in discussions that have already dragged on for two years. The present government had commissioned a group of experts to review the existing regulations, the *Hochschulrahmengesetz* (HR6) and suggest improvements. These regulations were themselves a delayed response to the student unrest in the late 1960s, and had required four years of complex negotiation before coming into force in 1976 under the then Social Democrat/Liberal government.

One important feature of the earlier proposals was to shift the balance within university committees to a wider group, making it possible for professors to be outvoted by the combined representation of students, assistants and non-academic personnel when they are not unanimous among themselves. The plan to return to the former system, supported by the Education Minister Dorothee Wilms (Christian Democratic Party, CDU), is the main reason for the students' protest, although they also object to the proposed introduction of a two-tier system of studies and the relaxation of rules for industrial research within universities.

The amendments would allow particularly talented students to take additional courses while the majority would have to complete their studies after six semesters. Critics feel that this would divide graduates into two groups, one of which would pick up the good jobs while the other would have to compete for an ever-diminishing number of academic openings. And the planned relaxation of the regulations covering research paid for by other than official sources, usually by industry, has created intense mistrust.

Minister Wilms is becoming increasingly isolated, as the students are no longer alone in their protests. Even the West German Conference of University Rectors (WRK), once the prime mover of reform, has distanced itself from the present proposals, which it believes go too far. It is even being asked whether amendments are necessary after all. Meanwhile, the education minister is losing further ground: her hopes that the new regulations would be passed before the summer parliamentary recess have been crushed.

Even states governed by CDU have started to question the need for a change. The chief minister of Rheinland-Pfalz, Bernhard Vogel, has declared that the present regulations "are better than their reputation", on the grounds that they have enabled the universities to recover from the crisis of the 1970s. This reflects what most state governments are think-

ing.

The *Länder*, which would have to implement such new regulations as are eventually embodied in a new federal law, also feel that the change is unnecessary. Most have learned to live with the present law, with all its problems. This is exemplified by Bavaria, which has adopted a law to facilitate additional financing for research which is acceptable both to industry and the universities without having had to wait for changes in the federal law.

As time goes by, the opposition grows stronger. It is now uncertain whether the new proposals, even as amended, will be accepted by the *Bundestag* and the *Bundesrat* in the next parliamentary session. Speakers for the Social Democratic Party (SPD) have moreover declared that the plan of Dorothee Wilms to undermine the principle of the comprehensive universities (*Gesamthochschule*) is unacceptable, as is the proposed dissolution of the Study Reform Commission, a body concerned with the improvement of study courses. SPD even promises that the cancellation

AIDS

Blood test trials inconclusive

Washington

THE first actual performance data on the acquired immune deficiency syndrome (AIDS) blood test are now in and they are a mixed bag. The test became available to blood banks in the United States in March following an all-out effort by the Public Health Service (PHS) and five contractor companies to meet a six-month deadline imposed a year ago by Secretary of Health and Human Services Margaret Heckler.

The performance data suggest that at least some of the concern voiced by critics of the rush effort have still not been adequately addressed. Of the 593,831 units of blood tested and reported to PHS during the four weeks beginning 22 April, 0.89 per cent tested positive in the first instance for AIDS antibody. That rate is not inconsistent with earlier estimates of the prevalence of AIDS antibody in the general population. But only 0.25 per cent of the blood samples were "repeatedly reactive" which, according to the PHS definition, means that they test positive twice in a row by the test or two times out of three if tested three times. The tests now in use all use enzyme-linked immunosorbent assays (ELISA) to detect the presence of antibody to the AIDS virus, HTLV-III/LAV. The new data cover about 70 per cent of all blood collected in the United States during that four-week period.

Although the data say nothing about the false positive rate, one of the major concerns at the outset, the large number of samples that tested positive initially but

of the new regulations would have high priority if it were to return to power. After its recent electoral successes (see *Nature* 6 June, p.450), SPD has become very self-confident.

Second only to the students, the loudest protests have come from the universities themselves. Having come to terms with the present law, they feel that politicians should concentrate more on the real problems: the steady erosion of financial support, the fact that 1.3 million students are enrolled on courses originally planned for 800,000, the rapidly increasing trend to unemployment among graduates and the exceptionally discouraging prospects for promotion within the university system, where the available professorships were distributed too readily during the years of expansion.

Despite all the criticism, Minister Wilms is insisting on her plans. They may represent the only significant project available to her, especially if the lifetime of the present government is limited. But her proposals are becoming less and less acceptable as the debate continues. Yet if the new proposals are not soon made law, little will remain of their original obviously reactionary character. **Jürgen Neffe**

negative when rechecked, is a worry. Test kits produced by at least two of the five companies, Abbott and Electronucleonics, showed wide variations of activity at first; Electronucleonics even had to recall from blood banks one early batch of kits on this score. Although improved quality control has done much to correct the problems, some variability apparently remains.

Meanwhile, at least some of the false positive reactions have been explained. People with certain HLA antibodies appear to react consistently positive to at least one of the tests on the market. It may be that because the test kits use virus antigen grown in human cell lines, some of the HLA antigens found on the human cell membranes are picked up in the purification of its virus protein. This problem may be eliminated when test kits derived from genetically-engineering virus antigen (grown in *Escherichia coli* rather than human cell lines) become available. These are being developed by Genentech and Centocor; clinical trials are expected to begin within two months.

So far, no data are available on how the ELISA test kits' performance compares with independent (and generally more reliable and more expensive and difficult) tests such as the Western blot. Some initial data will be presented at a conference at the National Institutes of Health on 31 July. These results should provide the first accurate reading on the false positive problem. **Stephen Budiansky**

Eureka

France puts flesh on bones

Paris

EUREKA, the French-inspired programme to unite European high-technology industry behind some concrete, profitable and advanced joint enterprises, has a public shopping list at last. Last week CESTA, the Centre d'Etudes des Systèmes et des Technologies Avancées, a public agency with an office in the very courtyard of the French ministry of research, produced a 70-page document listing five "priority action programmes" for Eureka. It is the product of two and a half months of intensive effort by the small and now somewhat fatigued interministerial Eureka group within the French government.

Until the European conference of research and foreign ministers in Paris later this month, the CESTA proposals are the nearest to a bible of exactly what Eureka is — or could be. Only "some" of the proposals are the result of recent shuttle diplomacy; the rest spring from unilateral French imagination.

The CESTA report defines five priorities: computers, networks and communications, robotics, biotechnology, and materials. And in each, it concludes with specific projects, timetables and potential European partners.

Computers dominate the proposals. In a programme dubbed "Euromatic", the Eureka group suggests that Europe should develop by 1992 a "super-Cray", capable of the 30 GigaFlops (floating-point operations) per second "required in the design of complex systems". It should be vectorial, use gallium arsenide chips (as yet unperfected technology), and could link the French companies Bull (once Honeywell-Bull) and Thomson with Siemens in West Germany. Although the market for such machines may be small, they are becoming so "essential" in certain key applications that Europe should develop a strategic independence in the technology from the present US and, shortly, Japanese suppliers.

Also under "Euromatic", the French group identifies a need to study "highly parallel" computer architectures aimed at a machine of 10 GigaFlops by 1992, via a demonstration machine 100 times less powerful by 1988. This would involve collaboration between Bull, Thomson and the German computer company GMD, which would "form the core of a broader European team including other partners (for example Britain, Italy)".

Other Euromatic projects include the development of high-capacity mass memory, starting with "giant" magnetic disks and moving on to optical disks (Bull, plus BASF and Siemens of West Germany); a software engineering centre (five companies in France, four in West Germany plus Aachen University, Philips in the Netherlands and ICL, Logica and Scicon

of Britain are suggested); artificial intelligence applied to "four precise projects" (nine companies and one university in France, four companies in Britain, Olivetti of Italy, Norsk Data of Norway and Philips and the University of Amsterdam could be involved); and there would be work on synchronous architectures, dedicated circuits and symbol processors.

The prospectus also lists projects on expert systems, multilingual access to databases, the management of large-scale industrial processes, a European micro-computer "paving the way to advances in integrated circuitry and the image processing industry, now a US monopoly", a 64-megabit dynamic memory and a European development unit for gallium arsenide technology and custom circuits.

Euromatic is by far the most specific of the five "priority actions". In robotics ("Eurobot"), France proposes the development of intelligent semi-autonomous robots for repair and maintenance in hostile environments (such as nuclear reactors), an entirely automatic agricultural tractor (Renault, Fiat and Saab could be interested), flexible-production factories (using computer-automated design and manufacture) and carbon dioxide, carbon monoxide, excimer and free-electron lasers as machining tools.

"Eurobio" involves the production of new crops and varieties for the seed industry, and the development of crops "as a means of transforming industrial processes"; and the research and development of systems for the control and regulation of biochemical processes (including biochemical reactors and novel means of drug delivery). The objective in the seed industry would be to give Europe "a leading position" on the world seed market, estimated to be worth some \$10,000–12,000 million, and some two dozen European companies, institutes and universities are identified as potential partners.

Add to these programmes "Eurocom" (communications) and "Euromat" (the development of a ceramic turbine) and you have a list of most of the high technology challenges facing not just European but all developed-world industry. So what is special about Eureka? First, the sense of urgency. Second, the fact that even the ideological French have discovered pragmatism; they say here that "nothing is fixed" about Eureka except that some practical actions should be under way by the end of this year. The key is probably government backing. "We don't expect governments to be coming with their chequebooks" to the Eureka conference later this month, they say, "but we do want to define the structure of financing". Which means it is time to say who will pay, and how.

Robert Walgate

UNESCO

Reforms now under way

Washington

A SERIOUS attempt is being made by the United Nations Educational, Scientific and Cultural Organization (UNESCO) to answer criticisms by Western nations. The executive board, meeting in Paris, has urged strengthening UNESCO's scientific and environmental activities, which are generally well thought of, but also put limits on more controversial programmes such as "Communication in the service of man". US officials seem pleasantly surprised by reports of the meeting but stress that no immediate decision to rejoin is on the cards.

Decisions taken at the marathon six-week meeting in Paris are not binding on the organization; they will however be put to the UNESCO general assembly next October in Sofia, Bulgaria, where all 260 member countries will be represented. The most pressing task has been to plan a 1986–87 biennium budget that is 25 per cent lower than the previous biennium, due to the withdrawal of the United States. Western nations (particularly Britain, which has also threatened to withdraw unless there are improvements) wanted to see the selective elimination of programmes that were judged to be either wasteful or opposed to Western interests; the Soviet Union, on the other hand, wanted to see a 25 per cent reduction across the board. So far, the West seems to have had its way.

The communications programme, for example, has been made uncontroversial by strictly limiting its more theoretical aspects, while devoting most of the funds for this topic into training of journalists. The Intergovernmental Programme on Man and the Biosphere, the International Geological Correlation Programme and the International Hydrological Programme, on the other hand, have been recommended for strengthening.

There has also been a reduction in the number of subprogrammes in the draft budget (another Western bugbear), and the whole plan has been presented in a new format that breaks with convention by being relatively easy to understand. Cost-benefit analysis is used explicitly, apparently for the first time.

The executive board has essentially ended up with a zero growth budget, with all activities beyond those that can be paid for after the 25 per cent budget reduction placed in a special category. The board suggests that similar planning should be adopted if Great Britain and Singapore do withdraw after the next assembly, as threatened. The changes so far at UNESCO, though pleasing to Western eyes, are not considered likely to make Britain change its mind.

Tim Beardsley

French telecommunications

National data network crashes

Paris

THE technical revolution in the French telecommunications system has been temporarily stopped in its tracks.

After being transformed from rustic to semidigitized in ten years, the change has been so great that it has entered the language. To be fashionable in France these days is to be "*cablé*", or "wired-up". Last year, it was "*branché*" ("plugged-in"), or even better the transposed "*chébran*".

But now and for two months, Transpac, the French and the world's most-used packet-switched data network, will be out

of bounds to individual users. Last week, private users so overloaded the system with their messages and consultations of electronic newspapers now on full-text database that the whole network crashed.

The cause is a bug in Transpac software for assembling and disassembling data packets, the groups of a few characters of a message which are given heads, tails and an address and then sent independently through the network so as to make efficient use of the gaps in other messages. "Packet assembler-disassemblers" (PADs) using this software are distributed throughout Transpac, and are designed to shut down when overloaded. At the highest levels of use reached last week, however, one PAD shut-down caused such an increase of traffic through others that they also shut down, leading to a chain-reaction which closed the whole of Transpac almost instantly.

This may have irritated the 800,000 or so home-users who now have access to Transpac through their free "Minitel" terminals, distributed by the telecommunications authority, PTT, to replace printed telephone directories. But it was disastrous for the banks, airlines and other companies with high data traffic that now rely on Transpac for business. Hence the decision by PTT to unplug the poor *cablé* public, until September, by when the Transpac bug should be sorted out.

Meanwhile the free distribution of Minitels (simple screens and keyboards) has now been suspended, but earlier projections were that the number of *Minitelists* would more than double to 1.7 million this year, and perhaps reach 3 million by 1987. Transpac could cope with this if it were not that *Minitelists* are not behaving as expected.

The typical "*tapeur*" at the keyboard does indeed consult the few hundred databases available — that much was expected — but, at least until the great crash, he or she spends much more time sending electronic messages to other users through systems such as "Missive", an electronic mailbox supported by PTT. And a little arithmetic shows that while 3 million times a few hundred databases is a large number of connections, 3 million squared is huge, probably too huge for even Transpac to cope with it.

That is why there is now some concern that the apparently logical French approach to create a single giant data network may not be so logical after all. For while the roads and rail seem capable, on the whole, of carrying both goods and people, it may turn out that personal data habits and the needs of industry (transferring large files with infrequent calls on a few trunk routes) are so different that quite different networks and strategies will be needed.

Robert Walgate

University of Geneva

Karl Illmensee resigns

DR Karl Illmensee, professor of biology at the University of Geneva, has resigned his post with effect from 30 September 1987, the date on which his present contract would normally expire. Illmensee's decision was submitted some days before a meeting of the professors of the University of Geneva called for 1 July at which the faculty was to have decided whether or not his appointment should be extended. The meeting would have had before it a report on the events of the past two years, during which the validity of some of Illmensee's published work has been questioned.

This latest development is probably a consequence of the report's recommendation that Illmensee's appointment should not be renewed. The report was prepared by a faculty commission appointed last February under the chairmanship of Professor R. Epstein, of the University of Geneva. Professor Illmensee had already been asked to provide a document giving his comments on the events of recent years, which is analysed in the report prepared for last week's faculty meeting. In the event, the matter was not discussed.

The Epstein report says that the quality of Illmensee as a teacher is not disputed, but that there are reservations about his diffidence in sharing his techniques with collaborators. Without "wishing to give too much weight to the matter", the report also finds that Illmensee had over-stated his wife's qualifications for her work as an assistant in his laboratory. Neither of these matters was taken up by the international commission looking into the more serious charges against Illmensee which reported in February last year.

Of the charges that some of Illmensee's published work is false, the report says (in respect of a series of experiments in which parthenogenetic mice were born) that there is no objective basis for proving Illmensee's observations wrong, but that it is a fair complaint that he had made no attempt to repeat the experiments.

The faculty commission makes the same complaint about Illmensee's failure to repeat the experiments involving the transplantation of nuclei into embryonic cells. Dealing with Professor Illmensee's unpublished defence, examined by the Epstein commission, the report says it does not accept his contention that he lacked the time and the means to repeat the experiments.

The commission explains its unwillingness to follow the report of the international commission by saying that that report was more damaging than its failure to find proof of deception would have suggested. The Epstein report also says that repetition of the disputed experiments was a key recommendation. □

Charity for MRC

A MAJOR new institute is to be set up within the University of Oxford to apply molecular biology to medicine. The British Medical Research Council (MRC), which has been the prime mover in founding the institute for Professor David Weatherall but which has not had the cash to do so, has finally enlisted the aid of the Wolfson Foundation to the tune of £1 million. Building should take two years.

The object of the provisionally named Molecular Medicine Institute, which will be on the site of the John Radcliffe Hospital in Headington on the outskirts of Oxford, is "to create a critical mass of good science and scientists within an essentially clinical environment", says Weatherall. Each of the five or six groups within the institute will be fully integrated into the clinical school and will need to be self-sufficient in research money. MRC and Wolfson have provided the capital costs, with help from the university and the Cephalosporin Trust.

For the Wolfson Foundation, this is the first formal collaboration with any research council and the case, says Wolfson director Dr J. Black, was simply considered on its merits, which were considerable, according to Sir James Gowans, secretary to MRC. "Our collaboration is an example of the council not necessarily being resource-led. When something is important enough that it has to be done and government policy means that the resources are not available, it is a question of finding help."

Neither partner will agree that Wolfson bailed out a previously committed MRC, but Black says there has been no discussion of a continued collaboration between the two and any new request would be treated on its merits. With the present financial state of the research councils, it seems likely that they will nevertheless look more eagerly at the £7 million Wolfson disbursements each year, mainly for the capital cost of constructing new laboratories.

Peter Newmark

Swedish security

Too much free information?

Stockholm

THE Soviet Union has denied using the international interlibrary loan system to obtain sensitive defence-related information from Sweden. A spokesperson of the official Soviet news agency APN pointed out last week that, if the Soviet Union did indeed require such information, it could acquire it by a "clearer and safer method", using satellite photographs. The Swedish defence staff has, however, launched a special inquiry into possible leaks through the interlibrary loan service.

International loans from Sweden are handled by Lund University Library, one of the two Swedish copyright deposit libraries that receive a copy of every work published in Sweden. (The other is the Royal Library in Stockholm.) The Soviet Union borrows between 750 and 1,300 works from Sweden each year, mostly in the humanities. So far, only a handful of "sensitive" items have been requested, all so far from "open" unclassified literature. To refuse to send such items would run counter to Sweden's policy of open access to information, and would require the special intervention of the government. This, according to Nils Palmborg, the Lund librarian in charge of the loan service, is hardly likely at the present moment.

According to Palmborg, Lund was first

alerted to the possibility of the loan service being used for non-academic ends some three years ago, when an East German naval library requested a copy of *Marinnytt* (Marine News). This is in fact readily available from all Swedish embassies and cultural institutes, and the main puzzle about the request was why the East German navy should bother to use the loan service when they could simply have picked up a copy in East Berlin. The Lund librarians duly forwarded the copy, but felt it worthwhile to inform the Swedish defence staff and to keep a watch for further puzzling requests.

In autumn 1984, they received a request from the Soviet Union for details of naval harbours in Norrland (North Sweden) and of a hydrographical survey of the same region. Later came a request about an area of central Skania (southern Sweden) where there are several military installations. According to Bertil Lagerwall, chief press spokesman of the defence staff, the material requested in the latter case included maps, photographs and also details of new building works in the area, all of which would be of considerable interest to anyone planning an airborne invasion.

According to Palmborg, the Lund library has worked out an arrangement with the defence staff about such requests.

Lund will forward the material to the library filing the request (in the case of the Soviet Union, the Lenin Library in Moscow), and will then notify the defence staff of what has been sent. The rationale here seems to be that, since the defence staff is notified only after the material has been sent, the arrangement does not compromise Sweden's commitment to the free flow of information. There does not seem to be any overt international agreement that requests for the transfer of information should be a matter of confidence between the parties concerned, and the Swedes presumably feel that, if the motive behind the request is innocent, the person or organization filing it would have no valid objection to a third party (in this case the defence staff) being informed.

For whatever reason, both Lund and the defence staff kept quiet for several months about the agreement, which was worked out last February. It was first revealed by the *Sydsvenska Dagbladet* two weeks ago and evoked considerable public comment and concern. According to defence spokesman Lagerwall, however, the Swedish government's commitment is clear: "We cannot and will not censor open literature", he declares. **Vera Rich**

Hans-Otto Wüster

THE European thermonuclear fusion project suffered a serious blow at the end of last month, with the unexpected death of Dr Hans-Otto Wüster, the director of the JET (Joint European Torus) project. Dr Wüster (58) was a West German theoretical physicist with a PhD from the University of Köln, where he worked for seven years at the Institute of Theoretical Physics. Thereafter, he spent successive spells



at the Deutsches Elektronen-Synchrotron (DESY) at Hamburg and at CERN, Geneva, where he was deputy-director between 1971 and 1975.

Dr Wüster was appointed director of JET in 1978. During his time, the torus was commissioned successfully. Much of Dr Wüster's contribution during his spell as director was to ensure political support for JET. But the laboratory says that he will also be much missed for his strong but sensitive style of management. □

Giotto now on target for Halley's comet

THE Giotto spaceprobe, the European Space Agency's contribution to the fleet of six craft aimed at Comet Halley, was successfully launched by an Ariane 1 launcher from Kourou in French Guiana last week. The three-stage launcher placed the satellite in a geostationary transfer orbit, from which an on-board motor injected the craft into a heliocentric trajectory aimed at an encounter with Halley on 13 March next year.

One important aim of the mission is to enter the coma of the comet and, using a four-colour camera, obtain images of the nucleus, possible only from within the coma. The best resolution hoped for is 50 metres. The composition of the coma itself will be sampled by neutral and ion mass spectrometers and dust detectors, while

the electromagnetic environment will be probed by magnetometers and plasma analysers. The spacecraft is not expected to survive beyond its closest approach to the nucleus of less than 500 kilometres due to the effect of dust impacts — its relative speed is expected to be 68 kilometres per second.

These experiments will to some extent be complemented by those aboard the five other spacecraft that should encounter Halley at about the same time (see table). Giotto's instruments will be switched on in September or October. In particular, the plasma analysers will be used to sample the solar wind at the time of the encounter of the International Comet Explorer with the comet Giacobini-Zinner on 11 September.

Philip Campbell

Probes to Halley

Mission	Launch date	Encounter date	Closest approach	Nationality
Giotto	2 July 1985	13 Mar. 1986	500 km	Europe
Vega-1	15 Dec. 1984	6 Mar. 1986	10,000 km	USSR
Vega-2	21 Dec. 1984	9 Mar. 1986	10,000 km	USSR
Sakigake	8 Jan. 1985	11 Mar. 1986	7,000,000 km	Japan
Planet-A	14 Aug. 1985	8 Mar. 1986	200,000 km	Japan
International Comet Explorer	22 Dec. 1983*	28 Mar. 1986	32,000,000 km	USA/Europe

*Originally International Sun Earth Explorer-3, currently set to encounter Comet Giacobini-Zinner followed by Halley.

NERC's infrastructure defended

SIR—I wish to respond to the letter from J.S. McPherson (*Nature* 13 June, p.536) commenting inaccurately on the role of Natural Environment Research Council (NERC) HQ and NERC Scientific Services (NSS). The staff numbers he quotes are correct, but to say that "... NSS is largely administrative in composition" is wrong and grossly unfair to the 90 per cent of NSS staff who are scientists and technologists providing support for the research carried out by both institute and NERC-supported university scientists. Most of NERC's administrative staff are in the institutes.

A more than superficial perusal of NERC's annual reports for the four years 1980/81–1983/84 would reveal that in that period the "quality" of computing provided to institutes has increased by a factor of four, new and sophisticated marine

equipment has been brought into service, an image analysis facility has been brought into use (and incidentally attained a reputation sufficient to attract a Principal Investigator award from the National Aeronautics and Space Administration) and an instrumented aircraft made available for NERC-supported science. And all this has been achieved with staffing that is sensibly constant over the period.

Turning to the question of commissioned research, NSS has not, as a matter of policy, sought to sell its services in the market place. What is clear from the annual reports is that many of the contracts obtained by the institutes would not have been possible without the computing, the ships, the cartography and other support services provided by NSS and administrative services of HQ. It is regrettable that Mr McPherson does not

seem to have the text of NERC's annual report which describes the vital support role in which NSS staff are engaged.

Mr McPherson's letter contains other errors. He says that it is sad that 1,000 scientific jobs are to disappear. The NERC corporate plan published last February indicates that 900 (not 1,000) posts may be lost over the next five years. Staff reductions will be in all categories—scientific, technical and administrative—with the aim of maximizing scientific output in relation to available income. NERC would prefer not to have to reduce staff numbers but it has to be realistic, live within its means and provide its scientists with the equipment and support services which they need to be effective.

J. C. BOWMAN
Secretary

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The science of sea-dumping

SIR—We wish to comment on the independent review of the practice of dumping radionuclides in the oceans, commissioned by the UK Department of the Environment, carried out by a group under F.G.T. Holliday and published last year¹. The terms of reference were to "review the scientific evidence, including the environmental implications, relevant to the safety of dumping radioactive waste at the designated North Atlantic site". The report gives a detailed description of the history of dumping and of the structure and operation of the model developed by the National Radiological Protection Board (NRPB) and the Ministry of Agriculture, Fisheries and Food (MAFF)^{2,3} which is used to assess the environmental effects of disposal.

Scientific modelling should clearly specify the model in terms of the underlying theoretical assumptions, and should indicate specific tests by which the model can be validated.

In the Holliday review, we find little discussion of the theory and none of experimental validation. The review notes that "the modelling exercise is complex and involves hundreds of man-hours of work which it has been impossible to check", but concludes that "the science being undertaken in the United Kingdom is of the highest quality. The results are published in the open literature and are openly discussed at international meetings, and this provides some safeguards on the reliability of the work."

Thus it is proposed that the ultimate guarantor of the quality of science being undertaken lies in some form of international peer review. We do not wish to comment on this process as it affects the MAFF/NRPB model as a whole but the part which concerns biological pathways.

The biological pathways sub-model is a crucial component of the whole because it concerns the potential concentration of hazardous materials which are otherwise dispersed in the ocean. In the MAFF/NRPB model the trophic level concept⁴ is used as the basis for calculating the concentration of radioactivity in marine life. Yet after the work of the International Biological Programme (1964–74) it became clear that although trophic level one (green plants) could be identified and trophic level two (herbivores) could be found in terrestrial systems, the remaining "trophic levels" could not be adequately identified⁵. This made the concept untestable and therefore suspect. Current concern about the inapplicability of the trophic level concept to aquatic ecosystems is shown by Ulanowicz and Platt⁶.

In the MAFF/NRPB model, it is assumed that the main food chain can be modelled by six trophic levels plus humans. Misleading conclusions can arise from this view that the food chain is limited in length. Materials cycle through the food web by organisms investing faeces and carcasses, creating food chains which are in trophic level terms indefinitely long^{7,8}.

Furthermore, science is surely about testing models, not simply asserting them, however authoritatively. The Holliday review illustrates several *predictions* about the effects of sea-dumping, but does not contain a single experimental result or reference. It is claimed that the model has been "adjusted" or "forced" to reproduce certain physical features of ocean dispersal, though this in itself does not imply predictive value. The final result of extremely long-range forecasting, involving release from dumped containers, oceanic dispersal and passage through food

chains, is a predicted radiation dose to humans over the next 100,000 years (peaking at 100 years). A minimum requirement for the credibility of such predictions is experimental validation in some simpler case. For example, earlier sea-dumping (1950–63) was carried out, without containment, in the English Channel; there has been the possibility of 30 years' monitoring of this more accessible release for comparison with predictions. Furthermore, much data have been collected on contamination of the Irish Sea, which could also be compared with model predictions. Are there no results of validation studies? What would the Committee on Safety in Medicines think of predictions not backed up by clinical trials?

The Holliday report does prudently recommend that sea-dumping not be resumed "until the current international reviews and the comparison of sea-dumping with land-based alternatives have been completed". It is essential that such reviews should address the two issues—the theoretical basis of the model and its experimental validation—that are absent in the current report.

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Measuring the unmeasurable

After more than a century, the reality of Maxwell's displacement current has been demonstrated by direct measurement. So, too, but less conclusively, has been the Aharonov/Bohm effect.

ONE of the big surprises of Maxwell's theory of electromagnetism was his introduction of the concept of displacement current. By Maxwell's time, it was generally understood that an electrical current in a conductor would generate a magnetic field (Ampère's law applies). In modern language, the relationship is that the curl of $\mathbf{H}$, the magnetic field strength, is equal to the current, or that $\nabla \times \mathbf{H} = \mathbf{J}$, where $\mathbf{H}$ is the field and $\mathbf{J}$ the current, both of them vector quantities. Maxwell's innovation was to add to the right-hand side of the equation the rate of change with time of the electrical displacement, $\mathbf{D}$, related to the electric field strength by $\mathbf{D} = \epsilon \mathbf{E}$, where ϵ is the scalar permittivity of the material, itself related to the dielectric constant by a numerical factor depending only on the choice of units. Now the displacement current has been measured for what appears to be the first time.

None of this implies that the reality of the displacement current has been in limbo in the interval. Indeed, were it not for the displacement current, it would not be possible to deduce from Maxwell's equations that electromagnetic waves have the properties of light. Maxwell was well aware of the difficulty he was creating. Indeed, D.F. Bartlett and T.R. Corle from the University of Colorado at Boulder, who have now measured a displacement current directly (*Phys. Rev. Lett.* **55**, 59; 1985), quote him as describing as one of the "chief peculiarities" of his thesis "that the time derivative of the electric displacement must be taken into account".

The principle of the measurement is a good deal simpler than the practice. The obvious first step is to create a reasonable source of displacement current. What could be simpler than the steady increase of electrical displacement between the plates of a capacitor during the process of being charged, most simply by connecting its plates to a physical electric current?

Bartlett and Corle point out two obvious snags. First, the voltage would quickly reach the point at which there would be a spark between the capacitor plates. Second, since the obvious way of measuring the displacement current is to measure the associated magnetic field, the feasibility of the measurement is determined by the relative magnitude of the field and the sensitivity of available measuring devices. Bartlett and Corle point out that for realistic capacitors, the

expected magnitude of the magnetic field will be measured in micro-gauss.

From there, the design of the measurement is determined. The ideal of a direct-current measurement is unattainable, but a low-frequency current will do instead provided that the response-time of the ultimate measuring device is faster than the oscillation. The other requirement, that it should be possible to measure magnetic fields in the micro-gauss region, points uniquely to a SQUID (for superconducting quantum interference detector). And that, inevitably, points to the source of the complexity of the measurement, the need to contain the equipment in liquid helium.

Bartlett and Corle use a capacitor consisting of a pair of circular plates 7.6 cm in diameter and separated by 1.22 cm. The magnetic field between them is measured by a coil 1.5 mm in diameter encased in a stainless steel tube which, at liquid helium temperatures, acts as a shield against stray electric fields. The SQUID itself, based on a Josephson junction, is enclosed in a superconducting lead shield. If the arrangement were strictly symmetrical, the magnetic field induced by the displacement current, at some given phase of the driving current, would be represented by concentric circular lines of force with the field strength increasing linearly from the centre to the edge of the plates and then decreasing inversely with distance from the axis of symmetry.

At least to a first approximation, the geometry is exactly like that of the magnetic field associated with a current in a linear conductor except that, now, the current is displacement current and distributed across the whole area of the plates.

The complications are horrendous. Magnetic fields from the cables carrying the current are an obvious nuisance, if only because the measurement must be made inside a liquid-helium Dewar vessel and also inside a shield against stray magnetic fields from elsewhere. (The shield is a spherical copper vessel coated on the inside by superconducting lead/tin solder and filled with helium.) The leads carrying current to the plates are bent so that their final separate approach to the capacitor is along the axis of the arrangement, and also that the magnetic field generated by the power supply can be measured simply by rotating the measuring coil through 90 degrees. The use of an alternating power supply has the advantage

of allowing the phase of the oscillation of the displacement current to be determined directly.

The only surprise is the precision of the result. A plot of the field strength against distance from the centre of the plates is indeed a straight line, whose slope is determined by the small scatter of points to within three parts in 100. The measured slope differs from that calculated from the dimensions of the capacitor and the other parameters of the measurement by less than five per cent, possibly explained by the intervention of a steel tube between the two plates.

Not all physical measurements of this kind are so unsurprising, demonstrations as it were of the ingenuity of which physicists are capable. A few weeks ago (*Phys. Rev. Lett.* **54**, 2469; 1985), Giorgio Matteucci and Guilo Pozzi from the University of Bologna reported a measurement designed to verify the reality of the Aharonov/Bohm effect, the quantum phenomenon by means of which the phase of the wave function describing a particle (such as an electron) should be affected by the electromagnetic potential of an electromagnetic field even when the particles are not subjected to physical forces.

One of the first demonstrations of the effect, in which Matteucci and Pozzi also had a hand, consists in arranging that a beam of electrons will travel on either side of a superconducting tube with a magnetic field along its axis in such a way that magnetic forces do not affect the electrons. Although the apparent physical tracks of the electrons are unchanged, their phases are altered by the presence of the confined magnetic field, and interference fringes produced as a result.

What Matteucci and Pozzi have now done is to repeat the experiment with a thin platinum wire coated on one side only with a thin layer of aluminium. Because of the contact potential between the two metals, the electrons see the wire as if it were an unsymmetrical distribution of electric charge, but because the wire is thin, there is no effective force on the electrons passing on one side or the other. But yet again, because in this case the electrostatic potential is different on one side of the wire from the other, the phases change and electron interference fringes are produced. Indeed, the pattern of the fringes can be changed as the wire is rotated about its axis which will seem yet another *tour de force*. **John Maddox**

Astronomy

An extraordinary gravitational lens and the redshift debate

from M.G. Edmunds

ONCE in a while a remarkable astronomical object is found in a fairly routine observing programme. Just such an object turned up last September during a galaxy redshift survey by the Harvard-Smithsonian Center for Astrophysics. The first published set of observations (Huchra, J. *et al.* *Astr. J.* **90**, 691; 1985) shows that right in the centre of an unremarkable redshift ($z=0.04$) spiral galaxy there sits the image of a high redshift ($z=1.7$) quasar.

There is no doubt that the quasar is of high redshift — the spectrum is almost a textbook example, showing strong hydrogen Lyman α and carbon-ion emission lines. The brightness and size of the galaxy (known by its positional coordinates as 2237+0305) are perfectly consistent with its low redshift. Yet the quasar is within about one-third of an arc of the centre, as judged on images recorded by a charged couple device solid-state detector when the terrestrial atmospheric seeing conditions were smearing point images to about two seconds of arc in size.

It is fascinating to speculate how the debate over the origin of quasar redshifts would have been affected if this object had been discovered twenty years ago, but it is unlikely that current theories of the non-cosmological origin for redshifts will have to be resurrected. Many cases are known of bright nuclei in galaxies that show quasar-like spectra (the so-called Seyfert galaxies) but whose redshifts (and by implication, distance) are the same as the host galaxy. The new object can probably be interpreted as a distant quasar, the image of which is brought towards us by the gravitational field of the nearby galaxy acting as a lens. The detection of the first gravitational lens (other than the classic deflection of light by the Sun) was reported in *Nature* in 1979 although they had been predicted by Einstein and championed by Zwicky in the 1930s. Five good cases of gravitational lensing of distant quasars are already known (for example, Turner, E.J., Ostriker, J.P. & Gott, J.R. *Astrophys. J.* **284**, 1; 1984), but these differ from 2237+0305 in that the galaxies causing the lensing are much more distant and they produce at least double quasar images.

The fun really starts when an attempt is made to estimate how likely it is that we should be able to find a system like 2237+0305. The assessment of *a posteriori* probabilities for a single object is, of course, a very subjective and uncertain task. Nevertheless, it is clear that this is a very unusual system. The chance of find-

ing such a bright quasar (approximately 17th blue magnitude) within an arc second of the centre of galaxies that have been spectroscopically surveyed would be only about one in ten thousand but, as with a conventional lens, the gravitational lens can not only bend light but also brighten the image. The difficulty, as pointed out by Huchra *et al.*, lies in the fairly large amplification factor that probably has to be invoked to increase the chance of finding such an object to, say, the few per cent level. If the quasar is not amplified, its intrinsic brightness (as implied by its redshift) must be unusually large.

Huchra *et al.* suggest that an amplification factor of 40 would give a reasonable probability of detection, and they claim that they can construct a lens model in which the required mass-to-light ratio of the galaxy is not unreasonably high (21 in solar units). But they neglect two factors in their revised probability estimate. The first is to fold into the estimate the probability of having a lens configuration that will produce such a high amplification; the configuration cannot be particularly common, since only very restricted conditions give high light amplification. The second problem is the effect of absorption by in-

terstellar matter in the galaxy through which the quasar is seen. Huchra *et al.* note that the optical continuum spectrum of the quasar appears to be highly reddened, showing a power law dependence on frequency of $\nu^{-3.5}$, compared with a typical $\nu^{-0.6}$ for most quasars. While it is possible that the object has an intrinsically steep spectrum, it is not difficult to show that the change in continuum slope from a typical value implies three magnitudes of extinction and hence requires yet another factor of 20 in amplification of the background object.

These two problems seem to make the detection probability dwindle too much for comfort, but it would be wise to wait for more detailed lens models and further observations before passing judgement. Higher spectral resolution observations reveal whether the quasar shows any sign of interstellar absorption features, such as calcium H and K lines and the sodium D line, which would give an independent estimate of extinction. Observations at deeper and higher spatial resolution, which will require seeing conditions of about half an arc second, may resolve the central image into subsidiary images which would allow better modelling of the lensing. The lensing explanation would, of course, be established were spectroscopic evidence that each of the split components had the same redshift to become available — an excellent project for the Space Telescope when it is launched. □

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Protein structure

A new twist for hairpin turns

from Jane S. Richardson

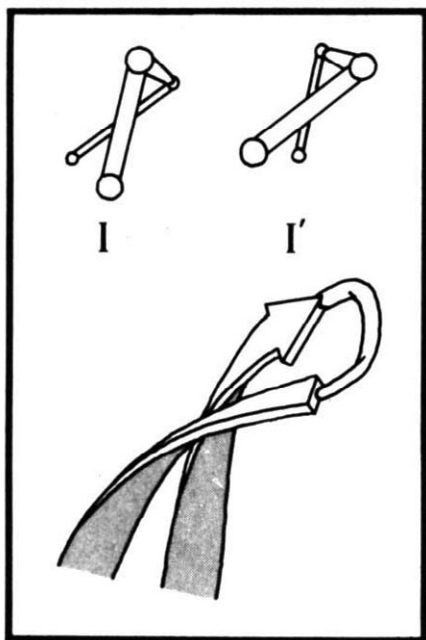
IN protein conformation, the tight turn^{1,2} is considered the classic, neat solution for connecting two antiparallel strands of beta-sheet. However, in the course of surveying and cataloguing short beta-hairpin connections (see page 170 of this issue³), Lynn Sibanda and Janet Thornton have unexpectedly found that this apparently obvious combination is not so well matched after all.

Beginning with the chiral alpha-carbons of natural L-amino acids, conformational features at all levels of protein structure show strong handedness preferences such as the right-handed alpha-helix, the right-handed twist of beta-sheet (see figure and ref. 4), and the right-handedness of beta-alpha-beta crossover connections^{5,6}. Tight turns also have a handedness, as they are not really planar as often drawn but have a pronounced dihedral angle defined by the four successive alpha-carbons. In the most common turn, termed Type I, that dihedral angle is approximately -45° (ref. 7). There is a mirror-image version of the

Type I turn (called Type I') which has a $+45^\circ$ dihedral angle, but it is very rare because it puts the side-chain beta-carbons uncomfortably close to the backbone and so only works well with glycine.

Sibanda and Thornton⁷ now show conformational preferences for each size category of beta-hairpins. For the shortest hairpins (with only two residues connecting the last hydrogen-bonded pair in the beta-strands) they find only 4 Type I turns but 15 Type I'. This is quite startling, as it reverses the overall occurrence statistics by a factor of about 40. The unusual circumstance responsible for that reversal is presumably a strong interaction between the twist of the beta-sheet and the twist of the turn. Those twists match perfectly for Type I' but fight each other for Type I, as illustrated in the figure.

One can imagine this built-in conflict as resolvable in two distinct ways during the folding of a real protein structure. If a beta-hairpin initially formed with its turn in the standard Type I geometry, the beta-



sheet twist would put a torque on the turn. In some cases the outcome could be breakage of the one or two hydrogen bonds closest to the turn, usually forming a kink in that part of the hairpin with the result that it would be classified by Sibanda and Thornton as a four-residue rather than a two-residue beta-hairpin. This outcome is more likely when the turn side chains are bulky, and is probably the only way to accommodate a proline. In other cases the torque might produce a shift of ϕ and ψ conformational angles over to values suitable for a different turn type. Types I and I' probably do not interconvert, as their ϕ and ψ angles differ by an average of 120° . However, there is another mirror-image pair of turn types available called II and II'. Type I could probably be converted to II' while Type II could switch to I', greatly improving the match to the beta-sheet twist in both cases. This sort of conversion should be much easier if one or both turn residues were glycine, and indeed 21 of the 25 short Type I' or Type II' beta-hairpins were found to contain at least one glycine.

The ubiquity of alpha-helices in proteins partly results from the fact that the local and long-range conformational preferences coincide. For beta-hairpins, however, we are now led to imagine the protein as arbitrating uneasily between the conflicting demands made by the turn residues and the beta-strand residues. □

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Geochemistry

Ocean islands – plums or plumes?

from Shen-su Sun

THE 'hotspot' model has played an important role in modern geological sciences ever since it was first proposed, more than 20 years ago, to explain volcanic activity in oceanic island chains, most notably the Hawaiian Islands. Morgan¹ suggested that hotspot volcanism is caused by melting of material that is welling up from deep in the mantle. Despite much research, leading to several competing models, a precise location for the sources of the zones of upwelling remains elusive. The situation presents a challenge to geochemists: can we develop tests to discriminate between the various models? In two recent papers Schilling and co-workers have addressed this question and provided firm support for the mantle plume model^{2,3}.

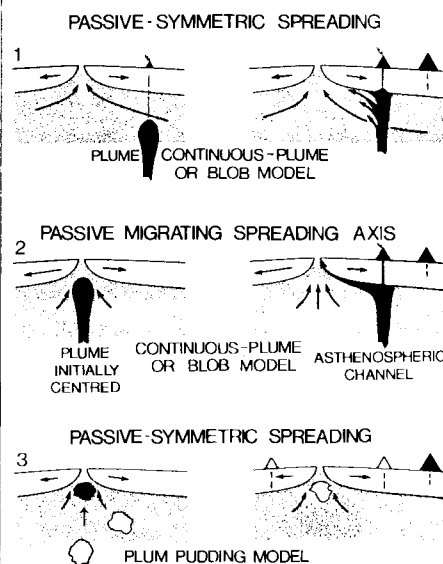
The arguments have centred on the geometric relationship between the mantle sources for hotspot volcanism and the sources for mid-ocean ridge basalts (MORB). The consensus view is that the source for MORB is located within the convecting upper mantle. The zones of upwelling judged responsible for hotspots, which Schilling *et al.* now regard as 'mantle plumes', seem to be essentially continuous and stationary in the convecting mantle with respect to the overlying lithosphere on a timescale of 100 million years^{4,5}.

Mantle plumes, according to Schilling *et al.*, are derived from 'fertile' regions capable of generating large quantities of basalt and located at depths greater than the depleted sources of MORB. Geochemical study of basalts from the Faroes-Iceland region led Schilling and Nygaard⁶ to suggest that upwelling of the plume material is cyclic on a timescale of 10–20 million years and in the form of 'blobs' instead of a continuous column. Alternative models include melting at the top of the upper mantle induced by propagating fractures caused by thermal and membrane stresses in the oceanic lithosphere⁷ or selective melting of fertile mantle domains of various sizes randomly embedded in the convecting upper mantle. The latter has been referred to as the 'raisin' or 'plum-pudding' model.

It has been known for some time that MORB dredged from 'normal' ridge segments well away from any hotspots is geochemically grossly depleted. Such depletion is characterized by low abundances of large-ion lithophile elements such as K, Rb, Th, U, Ba and Cs, together with low ratios of La / Sm and Nb / Zr, a high ratio of $^{143}\text{Nd} / ^{144}\text{Nd}$, a $^{87}\text{Sr} / ^{86}\text{Sr}$ ratio of about 0.7025 and a $^{206}\text{Pb} / ^{204}\text{Pb}$ ratio of about 18.0. In contrast, hotspot-related oceanic basalts are

generally have higher $^{87}\text{Sr} / ^{86}\text{Sr}$, $^{206}\text{Pb} / ^{204}\text{Pb}$ and lower $^{143}\text{Nd} / ^{144}\text{Nd}$ ratios. Basalts dredged from mid-ocean ridges near hotspots show a mixing character between these two types of source. This leads Schilling *et al.* to suggest that excess plume material rising beneath the ocean island is channelled along the mid-ocean ridge axis where it interacts with the 'normal' MORB². An ideal area for testing this channelling theory is in the South Atlantic where the ocean islands of St Helena, Gough and Tristan da Cunha lie 400–800 km east of the ridge axis. The ridge axis in the South Atlantic has been drifting westward relative to the hotspots at a rate of 1–2 cm per year for 25 million years. By detecting the geochemical effect of the plume material on the mid-ocean ridge system in MORB from the rift valley at latitudes corresponding to the extension of the hotspot traces, Schilling *et al.* provide convincing support for their hypothesis.

Consideration of the geometrical relationship between MORB and hotspot sources provides further evidence for the authors' theories. As shown in the figure, models 1 and 2, which combine the mantle plume model with passive feeding of the MORB source, can account for the observations. But as Schilling *et al.* point out, the chances that the plum-pudding model (model 3 of the figure) could generate the trace-element effect observed in MORB from part of ridge axes immediately opposite the tracks of near-ridge hotspots, and not elsewhere, must be very small. Additional support for the plume model



Schematic representation of mantle flow-plate motion models. 'White' volcanoes are derived from 'white blobs'; 'black' volcanoes from 'black blobs'. Modified from ref. 2.

comes from the observation that hotspot traces cross over ridge axes such as the Ninety east Ridge and Kerguelen Island in the Indian ocean and several hotspots in the Atlantic⁸. If the hotspots were generated by fracture propagation with their magma sources heterogeneously distributed at shallow levels in the convecting mantle, then the cross-over process should have erased all memory of thermal perturbation related to hotspots and, indeed, the hotspots themselves. Consequently, their source must be deeper than the immediate source region for MORB.

One possible snag with the proposals of Schilling *et al.* is that they require that divergent convective motion is inoperative under the ridge axis. It is not clear that this is the case. The possibility that advective overflow of the hot buoyant plume material along the connecting channel to the ridge axis could have been decoupled from the underlying convective upper mantle deserves further evaluation.

A by-product of plume material intrusion and entrainment in the depleted upper mantle is the creation of local heterogeneities — as envisaged in the plum-pudding type models. Consequently, plume and plum-pudding models need not be mutually exclusive in a dynamic mantle, which could very well satisfy geochemical and isotopic data observed in many isolated seamounts with no obvious connection to long-lived hotspot traces. That the Pb and Sr isotope compositions of basalts from Gough, Tristan da Cunha, Kerguelen Island and St Helena are unusual compared with most hotspots, offers the change of further quantitative evaluation of the plume-ridge interaction and the

plum-pudding formation process.

Finally, what about the source and cause of mantle plumes? Several models have been proposed, most of which are related to the fate of the subducted oceanic lithosphere⁹. If this is not smeared out by viscous flows and distributed back into the convecting upper mantle to generate ubiquitous heterogeneity, it could congregate into a distinct density layer, perhaps at the base of the upper mantle (about 650 km deep). Thermal disturbances in the deep mantle could then trigger density instabilities in this layer, inducing partial melting and plume upwelling. Such a subduction-revival cycle could take hundreds of millions of years. A spectrum ranging from pure 'chemical' to 'thermal' plumes could thus be generated according to the degree of mixing between the material derived from the subducted lithosphere and the depleted upper mantle¹⁰.

It is conceivable that a large plum within the slowly convecting mantle might be sufficiently robust to reach this deep layer where it could turn into a plume simply by adding an 'e' — where the 'e' stands for energy! □

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Endangered species

Salvaging single-sex populations

from Jared M. Diamond

LAST year I reported in these columns on the narrow escape from extinction of the Chatham Island Black Robin, which had declined to five males and two females (*Nature* **309**, 308; 1984). The rescue of the Dusky Seaside Sparrow (*Ammospiza maritima nigrescens*) poses a greater challenge, because the three surviving individuals are all males. A programme to salvage its gene pool, directed by Charles Cook and Herbert Kale (Kale, H.W. *Bird Conservation* **1**, 128; 1983 and *Florida Naturalist* **57**(4), 9; 1984), illustrates both the problems encountered in such efforts and the debates over whether the efforts should be undertaken at all.

The Dusky Seaside Sparrow, a local subspecies sometimes considered to be a full species, was formerly abundant in marshes on the east coast of Florida, but in the 1960s, most of its habitat was destroyed. During the 1970s the US Fish and

Wildlife Service (USFWS) spent five million dollars to save the sparrow by buying its remaining habitat, now the St John's National Wildlife Refuge. This programme failed because of the continuing destruction of the refuge habitat by fires and drainage. The last record of a female bird was in 1976. Finally, in 1979, five of the last six surviving males were brought into captivity for their protection.

At that stage, the only chance to salvage genes of the sparrow was to breed Dusky males first with females of the related Scott's Seaside Sparrow and then with successive generations of resulting hybrid female offspring. Five such crosses would yield birds that were, progressively, 50, 75, 87.5, 93.8 and 96.9 per cent Dusky. Once a large enough flock of 96.9 per cent hybrids had been obtained, some could then be re-established in natural Dusky habitat. To this end, a Dusky male and a

Scott's female were mated experimentally in 1980, yielding four 50 per cent Dusky hybrids.

For the next two years, while the last Dusky males grew older, USFWS refused not only to fund but even to permit the hybridization programme. When the service finally granted permission in 1983, funds still had to be obtained from private sources: these were given by Walt Disney World, Florida Audubon Society, International Council for Bird Preservation and Wildlife Preservation Trust. In 1983, one 75 per cent Dusky female was produced, followed in 1984 by another 75 per cent chick (not yet sexed) plus a 87.5 per cent chick that, tragically, broke its neck. As the 1985 breeding season approaches, the fate of the Dusky gene pool depends on the three surviving males approaching 10 years of age, which is very old for small temperate-zone songbirds.

This captive breeding programme illustrates vividly two biological difficulties: that small populations are very susceptible to accidents (such as the death of the chick); and that old birds are poor breeding stock. One of the old males has been unsuccessful at wooing females, and another yielded only infertile eggs. In 1984, the 50 per cent female produced eight clutches and twenty eggs, of which only five hatched and one survived. Thus, a practical message for salvage programmes is to start when there are many youthful survivors, rather than waiting until it is too late, or nearly so.

The broader question raised by the back-crossing programme is whether it is even worth attempting. Some of the objections raised by USFWS were baseless: that hybridization would dilute the Dusky gene pool (but it was doomed otherwise); that hybrids might not be fertile (they are); and that hybrids might not resemble the Dusky Seaside Sparrow (they do). However, another objection raises a significant issue: the Service neither wants to spend money on restoring Dusky habitat for eventual releases, nor on saving hybrids. (In the words of one official, "Legally, a 98.4 per cent Dusky isn't a Dusky".) Many biologists similarly object to captive breeding programmes on single species, when money for habitat preservation that could save many species is so scarce. However, the number of species for which captive breeding or back-crossing offers the sole chance of survival is growing rapidly and the current programme on the California Condor would have been impossible without the experience gained from similar programmes with the Andean Condor and Peregrine Falcon. Thus, although the Dusky Seaside Sparrow is just a small drab bird, the lessons being learned with it now will surely have wider application. □

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Gene regulation

Controlling roles for snurps

from Phil Turner

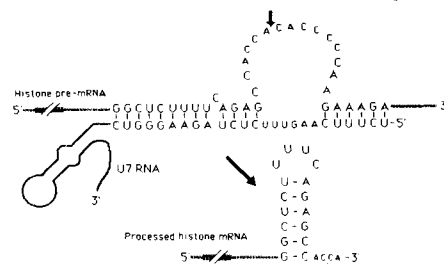
SEVERAL recent publications point to the possibility that small nuclear ribonucleoprotein particles, or snurps, may play much more important roles in the processes of gene regulation than previously thought. Over the past year or so, proof has been obtained of the involvement of snurps in the removal of introns (splicing) and the production of correct 3' ends from primary gene transcripts (pre-messenger RNA). Since there are now many examples both of alternative splicing pathways, which result in more than one mature mRNA species from a single pre-mRNA, and of the differential use of alternative poly(A) sites, snurps may not simply perform these processing steps but may be used to choose between the alternatives. If so, their action would constitute an important form of post-transcriptional control of gene expression.

Our best understanding of the biochemical reactions performed by snurps comes from the work of Max Birnstiel's group in Zurich on the involvement of the U7 snurp in the production of correct 3' ends of histone mRNA in the sea urchin. Based on the complementary DNA sequence of the sea urchin U7 RNA, Strub *et al.*¹ have suggested a model in which the 5' end of the U7 RNA hybridizes with two conserved regions at the 3' end of the histone pre-mRNA (see figure). The conserved regions in the pre-mRNA are the hairpin structure that is found immediately upstream of the 3' end of the histone mRNA in most species and a sequence (CAAGAAAGA) that occurs six nucleotides downstream of the actual 3' end of the mature mRNA. (The latter sequence seems to exist in an altered form in higher eukaryotes², possibly explaining the fortuitous requirement of the sea urchin U7 RNA for correct processing of the sea urchin histone H3 mRNA in frog oocytes³.) In this model the U7 RNA linearizes the hairpin structure by forming base pairs with 13 out of the 16 nucleotides in the hairpin. It also forms 6 base pairs with the CAAGAAAGA sequence and in doing so the region of the histone pre-mRNA between the conserved regions forms a small loop which is presumed to facilitate the endonucleolytic cleavage that produces the mature 3' end¹.

Support for this model comes both from the previous observation that the hairpin structure is absolutely required for correct 3' end production⁴ and from experiments recently reported in *The EMBO Journal*. Using a series of deletion and insertion mutants of the sea urchin histone H3 gene, Georgiev and Birnstiel⁵ have shown that the CAAGAAAG sequence is essential for pre-mRNA processing. These experi-

ments also demonstrate that the topology of the two conserved regions is important; the insertion of only six nucleotides between them abolishes processing. In addition, for the rate of processing to be maximal, the pre-mRNA must contain at its 3' end 50–80 nucleotides with no special sequence requirement⁴ and also a 5' cap structure⁶.

This picture of the processing of histone pre-mRNA by the U7 snurp has a number of similarities to the generation of the 3' end of other gene transcripts by post-transcriptional cleavage which, however, is then followed by the addition of a poly(A) tail. In an increasing number of mRNAs, additional sequences downstream of the essential AAUAAA sequence are found to be required for 3' end formation^{7,8}, with the poly(A) addition site falling between two required sequences. An analysis of 61 vertebrate poly(A) sites⁹ demonstrates that two classes can be described, depend-



Proposed role of U7 snurp in converting histone pre-mRNA into processed histone mRNA. Small arrow indicates the point of cleavage that generates the 3'-end of the processed histone mRNA. The two conserved sequence elements are shown in large letters. The histone coding region is the interrupted hatched line.

ing on whether the pentanucleotide CAYUG is immediately upstream or downstream of the site of poly(A) addition. Possible base-pair interactions of both these classes of poly(A) site with the U4 RNA sequence, which are similar to the suggested pairing of U7 RNA and histone pre-mRNA, have been noted⁹. Further evidence that snurps are involved in 3' processing of some polyadenylated RNAs comes from the evidence that certain antisera against snurps inhibit polyadenylation *in vitro*¹⁰.

The U4 and U6 RNAs form part of a single ribonucleoprotein particle as a result of intermolecular base pairing^{11,12} (which explains why U6 seems to react with the so-called Sm antisera despite lacking the sequence by which the Sm antigen is thought to bind). If the U4/U6 complex is involved in poly(A) processing then it must be dissociated in part for the U4 RNA to interact with the poly(A) site as proposed⁹. In this connection, the U7

RNA also seems to lack the sequence that would explain its precipitation with Sm antisera¹. Gel analysis of U7 RNA purified from a 12S snurp showed the presence of an RNA molecule about 100 nucleotides in length¹³. U5 RNA is approximately 100 nucleotides long and shares an 8-nucleotide homology with U7. The significance of these observations is not clear, but it is possible that certain snurps interact with each other during their processing activities.

Since the initial suggestion, based on sequence complementarity, that the U1 snurp might be involved in intron removal¹⁴, much supporting evidence has been obtained. Recently, Kramer *et al.*¹⁵ have shown that the ability of nuclear extracts of HeLa cells to splice pre-mRNA can be abolished by prior treatment of the extracts with human autoimmune antibodies and that the inhibition can be prevented by prior saturation of the antibodies with purified (U-type) snurps. In addition, splicing can be inhibited by removal of eight nucleotides from the 5' end of the U1 RNA of the snurp.

In a complementary series of experiments Tatei *et al.*¹⁶ have shown that U1 snurp fractions bind to synthetic single-stranded DNA 5' or 3' splice sequences or RNA transcripts derived from them, whereas U2 snurps do not. Further purification of the U1 snurp resulted in preferential loss of binding to 3' splice sites suggesting that additional components are required for U1 snurps to bind to both splice sites.

In vitro splicing of pre-mRNAs is most efficient if they possess capped 5' ends, the splicing reaction being inhibited by cap analogues as long as they are added no later than the start of the incubation¹⁷. The appearance of the spliced product, which occurs via the formation of a lariat or tailed-circular molecule (reviewed in refs 18 and 19), is only seen after a lag period of 30 – 45 minutes²⁰. Lag periods have also been observed for polyadenylation *in vitro*¹⁰ and for histone maturation *in vivo*; the latter is cap dependent⁶. The existence of a lag period in these processing events could indicate a requirement for assembly of pre-mRNA into a suitable RNA–protein complex or a requirement for a scanning process to ensure that only appropriate 5' and 3' splice sites come together. To test whether a scanning process is involved, oligonucleotides or antisense RNA transcripts that can form hybrids with introns or exons could be introduced into processing systems; scanning might be inhibited by short double-stranded regions, whereas folding of large introns to produce lariats should not be inhibited.

The argument usually put forward against a simple scanning model is that alternative pre-mRNA splicing pathways have frequently been observed, sometimes within a single cell type²¹ and sometimes with tissue specificity²². But scanning could still be operative if, before the

commitment to splicing is taken, certain criteria at the 3' side of an intron have to be fulfilled. These criteria would include the existence of a 3' splice-site consensus sequence and, in some cases, the existence of a branch-point sequence or functional equivalent in the appropriate position²³. For some introns, the adoption of specific secondary structures could be an added criterion²⁴, and the presence of another snurp particle bound to, or aiding recognition of, the 3' splice site may also be required^{16,19}. If the various criteria were not met, splicing would not take place and the criteria would then be reassessed at the next 3' splice site. In this way certain exons could be 'jumped' or processed out. This could occur stochastically within one cell type if two alternative secondary structures were possible for that intron but only one fulfilled the criteria, or if two types of 3' snurp particles were present but only one had the necessary affinity for particular 3' splice sites to allow splicing. In such a model it would be possible for the criteria for 3' splicing to be met by a different species of pre-mRNA molecule, giving rise to the process of 'trans-plicing'. With the processing systems now available we may not have long to wait to see if this kind of reaction can occur.

If there is scanning, pre-mRNAs that undergo mutually exclusive alternative splicing, such as the troponin T pre-mRNA²², ought not to be spliced *in vitro* following pre-hybridization to an oligonucleotide complementary to an exon sequence that is usually processed out. This is readily testable. In addition, for those pre-mRNAs which exhibit alternative splicing certain parts of the precursor sequence, intron or exon, must specify in some way whether or not the 3' splice site is to be used. This could be achieved through alternative secondary structures or by differential affinities for two classes of snurps, but for tissue-specific alternative splicing it is best explained by the binding of other protein factors, which influence whether a 3' splice site will be used. In this connection it has been reported that pre-loading the frog oocyte nucleus with certain ribosomal proteins can alter the processing of the pre-mRNA encoding that protein (I. Bozzoni, 10th EMBO Symposium, 1984). The processing of SV40 RNA in frog oocytes also suggests that different splice sites in the same pre-mRNA have dissimilar interactions with various snurp particulars during splicing²⁵.

Similar types of regulation can be envisaged where differential use of poly(A) sites occurs. In some cases the differential use of two sites may simply reflect the relative probability of the removal of an intron that contains a poly(A) site and the subsequent use of a distal site, rather than the occurrence of processing and polyadenylation at the poly(A) site in the intron, which would remove subsequent exons from the product. Which of these two pathways was used could reflect the

endogenous levels of the different snurp activities involved. This could explain the alternative use of poly(A) sites in the immunoglobulin μ gene transcripts²⁶. Tissue-specific use of alternative poly(A) sites would require additional trans-acting factors, which would presumably recognize sequence signals in the pre-mRNA concerned, and would vary the processing pathway taken by the snurp.

If snurps do perform some of these regulatory roles (with or without the assistance of other factors), then one would expect some classes of snurps to exhibit differential regulation, as has recently been noted for U1 snurps in *Xenopus*²⁷.

In conclusion, evidence is mounting that snurp-mediated processing events are important post-transcriptional regulatory steps for some pre-mRNA types. Perhaps when the functions of U2 and U5 snurps are finally worked out we shall have a better appreciation of the subtleties of this regulation. □

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Climatology

Impact of extreme events

from T. M. L. Wigley

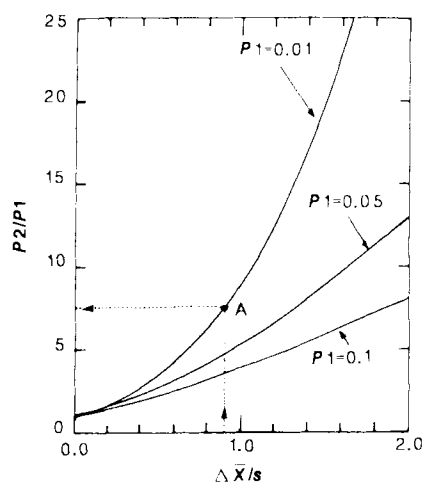
BECAUSE the impact of climatic change is so dependent on the society that is affected, it is difficult to isolate unifying features or rules by which to assess the impact. We can be fairly sure that the early stages of any worldwide change, such as warming caused by the CO₂ greenhouse effect, will not be a problem because socio-economic factors — diversity in agriculture, international trade and planned water resources — will act as buffers to change. Impacts accrue, however, not so much from slow fluctuations in the mean, but from the tails of distribution, from extreme events. In many cases, an extreme can be defined as an event where a climatic variable exceeds some absolute threshold. This has led to the concept of climatic marginality, first studied in detail by M. L. Parry¹ and now taken further by Parry and T. R. Carter².

In analysing the effects of the severe weather of the Little Ice Age on the peoples of southeastern Scotland, Parry originally made three points; that, on the margins of agricultural viability (through cold or dryness), extreme events must occur more frequently; that the frequency of such events might change dramatically as a result of even a small change in the mean climate; and that the probability of two successive extremes is even more sensitive to small changes in the mean. The importance of successive extremes lies in their amplified cumulative impact. A single extreme may be easy to withstand but, if buffer stocks are depleted by one bad season, a second in succession could be far more devastating.

The simple probabilistic aspects of

Parry's arguments are easy to demonstrate. Under a stable climate, an extreme event may be assumed to occur when, say, climate variable X falls below some critical threshold X_c . The probability of such an event is given by the area under the tail of the distribution where X is less than X_c . If we now shift the mean of the distribution towards X_c , this area will increase in a highly non-linear way, as shown in the figure. A change in the mean by only one standard deviation would make an extreme event that had been expected only once every twenty years become five times as frequently expected, and an event previously expected once a century would be expected every eleven years. For example, if the annual mean precipitation of about 920 mm over England and Wales fell by 100 mm (about 0.9 standard deviation) a drought that was expected one year in a hundred ($P_1 = 0.01$) would become roughly 7.5 times more frequently expected ($P_2/P_1 = 7.5$, point A in the figure). A doubling of CO₂ concentration is likely to produce changes of more than one standard deviation in both precipitation and temperature in many parts of the globe.

Changes in the probability of two successive extremes are even larger: two successive 1-in-20 year events go from being most unlikely (a return period of 400 years) to being quite probable (a return period of only 16 years). Of course, these are highly idealized calculations, but they do serve to emphasize the importance of extremes and provide a conceptual framework for estimating the impacts of future climatic change. Changes in both



Sensitivity of the frequency of extreme events to changes in the mean (based on an assumed normal distribution and constant standard deviation). Abscissa shows the change in mean (ΔX) as a multiple of standard deviation (s); ordinate shows the resulting change in the probability of extreme events with initial probabilities (P_1) of 0.1, 0.05 and 0.01.

inter-annual variability and in year-to-year persistence of climate may either amplify or dampen these influences of a changing mean. These ideas have been developed in more detail by Parry and Carter².

This focus on variability and extremes has been examined for shorter timescales by L. O. Mearns, R. W. Katz and S. H. Schneider³. Noting that some crops are sensitive to temperature extremes (for example, a few consecutive days over 95°F can significantly reduce corn yields in the United States they have used an autoregressive model to calculate the effects of a changed mean on the

frequency of daily extremes, specifically daily maxima over 95°F. The stochastic simulation approach they use is essentially that used in stochastic hydrology, where the technique has been developed to a high degree over the past 20 years. (At least one study⁴ has considered the impact of climatic change on water resources in this way, although the results were rather inconclusive.) Echoing Parry's conclusion, Mearns *et al.* find that the relationship between changes in mean temperature and changes in the probability of daily extremes is highly non-linear, and that small changes in the mean can sometimes produce relatively large changes in the probabilities of certain events. Their results are sensitive to assumptions regarding changes in variability and changes in day-to-day autocorrelation, but the qualitative conclusions hold even if these parameters are varied.

Changes in the frequency of extreme high (or low) temperatures are of importance not only to agriculture but also, for example, to energy demand and human mortality, so this type of analysis can provide broad insights into the way that climatic change may affect society. It also opens up new avenues for multi-disciplinary research into climate impact assessment. □

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Neuropharmacology

Super-potent serotonin blockers

from Leslie L. Iversen

STARTING ON page 126 of this issue, B. P. Richardson and colleagues from Sandoz describe the discovery of a novel class of serotonin antagonists which offer new insights into the pharmacology of this biologically active indolamine¹. The new drugs are among the most potent of any type ever described: some act at concentrations as low as 10^{-14} M. The compounds, which bear a structural resemblance to both serotonin and cocaine are indoletropanyl or homotropanyl esters. They specifically block the ability of serotonin to excite various peripheral nerves in the autonomic and sensory nervous systems. Furthermore, different compounds in the new series show wide variations in their potency for antagonizing the effects of serotonin on different types of peripheral nerve, suggesting that neural receptors for serotonin (termed M-receptors) comprise a variety of distinct

pharmacological sub-classes.

One important role of the M-receptors may be to mediate the painful effects of serotonin released during injury or vascular spasm, by triggering activity in the endings of pain fibres. One of the new antagonists, ICS 205-930, can block the painful effects of serotonin administered locally to human skin and may prove useful in controlling pain in migraine sufferers.

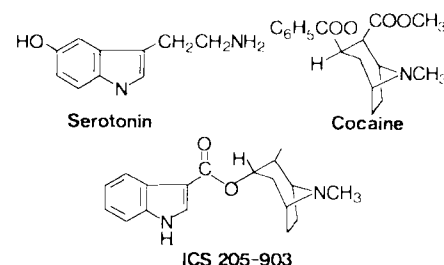
Serotonin (5-hydroxytryptamine, 5-HT) has been known for some forty years, and its pharmacological actions on various tissues have been studied intensively. Gaddum was first to show that the actions of serotonin on some smooth muscles can be blocked by the powerful hallucinogen D-lysergic acid diethylamide (D-LSD). A number of different receptor classes for serotonin has been suggested in peripheral tissues and in brain. Gaddum and Picarelli² showed that serotonin con-

100 years ago

THE COMET OF 1472. — M. Celoria of Milan has discussed the elements of the last comet observed by Toscanelli, which is the celebrated one of 1472, also observed by Regiomontanus, whose description of its path in the heavens enabled Halley to make a rough approximation to its orbit, as he states in his "Synopsis of Cometary Astronomy." The Chinese account of the comet's track contained in the supplement to the great collection of Ma Twan Lin, of which Edouard Biot published a translation in the appendix to the "Connaissance de Temps" for 1846, enabled Langier to make a further calculation of the orbit, though the somewhat full description of the comet's course amongst the stars is unfortunately very deficient in dates.

From *Nature* 32 231, 9 July 1885.

tracts the intestine by two different mechanisms: a direct action on smooth muscle, mediated by D-receptors, and an indirect action on autonomic nerves to stimulate acetylcholine release, mediated by M-receptors on neurones. More recently, radioligand binding studies have also revealed two classes of binding sites, termed 5-HT₁ and 5-HT₂. The 5-HT₁ sites are identified by high affinity binding of serotonin in brain and may mediate contractile responses in some peripheral tissues, particularly stomach. The 5-HT₂ sites, which may correspond to the D-receptors, are found in both brain and periphery; they mediate contractile responses in peripheral blood vessels and aggregation responses of blood platelets to serotonin³. These sites are potentially blocked by classical serotonin antagonists: cyproheptadine, cinanserin, methergoline, methysergide and ketanserin; in brain they are identified by using radiolabelled ketanserin as a ligand⁴. Both classes are potently blocked by D-LSD, although it is not known whether this explains LSD's unique psychopharmacology. Neither class is sensitive to the new Sandoz analogues, which appear to be specific for the neural M-receptors.



The development of the new compounds stems in part from the discovery by J. R. Fozard⁵, that cocaine is a weak but selective antagonist of neural responses to serotonin. Fozard and his colleagues at the Merrell Dow laboratories in Strasbourg used this discovery to develop a series of selective serotonin antagonists modelled on the cocaine structure. One of these, MDL 72222, reported last year⁶,

has a selectivity similar to the antagonists now described although it is considerably less potent. The Sandoz researchers first synthesized analogues of serotonin and discovered compounds that selectively mimicked its effects on neurones or on smooth muscle. This knowledge was used to synthesize antagonists with selective action on neurones in which the serotonin side-chain nitrogen is incorporated in a tropane ring, as in cocaine (see figure).

Interest in serotonin pharmacology has been particularly strong in the past few years. New generations of antidepressant agents are emerging that act by inhibiting the re-uptake of serotonin after its release from synaptic terminals in brain, thus enhancing its synaptic actions⁷. It has also been reported recently that novel anti-anxiety drugs of the buspirone type may act by selective stimulation of serotonin receptors in brain, although the precise category of sites involved remains unclear⁸. Receptors for serotonin of the

type that are blocked by the new antagonists have not yet been identified in the central nervous system but their presence there would not be surprising.

As so often in pharmacology, the discovery of novel drugs with selective antagonist actions will undoubtedly allow rapid progress to be made in defining the occurrence and functional significance of a hitherto obscure class of monoamine receptors. □

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Surface structure

X-ray reflection from liquids

from Stuart A. Rice

IN AN important paper in *Physical Review Letters*, A. Braslau *et al.* report measurements of the surface roughness of water deduced from the scattering of X rays reflected from the surface¹. To do so, they have introduced a new way to interpret the reflectivity data. This and related advances are beginning to revolutionize the understanding of liquid surfaces.

For two decades there has been a steady stream of improvements in the scope and sensitivity of techniques with which to study the atomic and electronic structures of surfaces. Many of these techniques use electrons as probes, or detect electrons, or both. Unfortunately, because electrons interact very strongly with the atomic charge distribution, the interpretation of electron-scattering data usually requires removal of the contributions to the signal from multiple scattering processes, which cannot at present be carried out for liquid surfaces. Unlike electrons, X rays are weakly scattered by the atomic charge distribution, and two X-ray techniques have recently been introduced to study surfaces: X-ray reflectivity as a function of angle of incidence, and grazing incidence X-ray diffraction. Both can be used to study liquids as well as solids.

That X rays will be totally reflected from a condensed medium if the angle of incidence is less than some critical value was predicted and first demonstrated by A.H. Compton in the 1920s². The thrust of the work in the following decade was concerned with the determination of the refractive index of matter at X-ray frequencies, assuming the sample to be homogeneous up to a planar surface, treated as a geometrical discontinuity. It was

not until 1954 that L.G. Parratt suggested inverting the analysis and interpreting X-ray reflectivity as a function of angle of incidence via models of the inhomogeneous surface-density distribution³. This suggestion lay dormant for twenty years, until B. C. Lu and S. A. Rice⁴ adapted it to provide information about the surface structure of liquid mercury. Other applications^{5,6} are rare.

X-ray reflectivity as a function of angle of incidence is sensitive to the distribution of density perpendicular to the surface; the greater the range of incident angles for which measurements can be made, the more accurate will be the inferences drawn about the surface density profile. The dynamic range required for such experiments is large — of the order of 10⁷ to 10⁸ — so synchrotrons are the favoured X-ray sources. The data can be interpreted by two means. In one, a model of the surface-density distribution is assumed and the predicted X-ray reflectivity is compared with that observed. Alternatively, the data can be interpreted in terms of some model-independent parameter that is characteristic of the density profile.

It is the second means of interpretation that has been introduced by Braslau *et al.* They show that the reflectivity data they obtain can be interpreted in terms of the mean square roughness of the surface without prescribing the molecular mechanism by which the roughness is generated. The value they obtain for this measure of the surface density profile is $3.24 \pm 0.05 \text{ \AA}$. A molecular dynamics simulation of a rigid charge distribution model of water⁷ shows that the density

falls smoothly through the liquid-vapour interface with a characteristic width of $3.56 \text{ \AA}$; the deviation between these values provides one measure of the inadequacy of the model potential used in the molecular dynamics simulation.

As expected, total external reflection of X rays is accompanied by fluorescence and, very recently, this has been exploited to study the properties of solid and liquid surfaces. Smirnov⁸ has shown that measurement of the fluorescence intensity as a function of angle of incidence of the exciting X rays can be used to infer the density distribution perpendicular to the surface — in his case, for a film of germanium deposited on glass. In an independent development, Bloch *et al.*⁹ have used the same kind of measurement to infer the concentration profile of a dissolved polymer in the solution-vapour interface.

To date, the only reported application of grazing incidence X-ray diffraction to a liquid is by J. Als-Nielsen and P. S. Pershan¹⁰ who measured the interplanar spacing perpendicular to the surface of a smectic-A liquid crystal. The interpretation of the X-ray diffraction pattern in terms of the in-plane distribution of molecules in a liquid-vapour interface is more difficult than for a solid, because removal of the contribution to the X-ray scattering from atoms below the surface requires knowledge of their distribution as a function of density. Whereas the atomic distribution in a crystal substrate is substantially the same up to the outermost plane of atoms, the variation in distribution in the 2–3-atom thick, inhomogeneous liquid-vapour interface is important. Nevertheless, guided by computer simulations, it should be possible to obtain useful information about the in-plane distribution of atoms and molecules in a liquid-vapour interface.

The availability of powerful tunable X-ray sources is likely greatly to increase our information base and understanding of liquid surfaces. A few of the possibilities we can expect in the next few years are studies of the structure of films supported on liquids and of phase transitions in those films; measurements of the influence of oxidation on the structure of the liquid surface; and investigations of the spatial distribution associated with the excess surface concentration of a component of a mixture. □

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Earth sciences

Calculating continental strength from thermal history

from Marcia McNutt

SEDIMENTARY basins in continental interiors contain economically important oil, gas and coal deposits. The development of such basins has been largely controlled by the thermal history and mechanical response to sediment loading of the underlying crust and uppermost mantle (the 'continental lithosphere'). N. Kusznir and G. Karner report on page 138 the use of rock deformation data from laboratory experiments to determine directly the mechanical properties of the lithosphere from the thermal history of a basin. In as far as the extrapolation of laboratory experiments to geological loading rates remains valid, their formulation provides a mechanism for inferring the thermal parameters that govern basin development from observations of the mechanical strength of the continental lithosphere through time.

As a first approximation, the long-term mechanical behaviour of the lithosphere resembles that of an elastic plate with strength characterized by its elastic thickness T_e . Rocks above the depth T_e are strong enough to support elastically the stresses imposed by the sediment, whereas rocks below that depth deform ductilely to relax stress. The elastic thickness is primarily controlled by thermally activated creep mechanisms and hence the temperature in the lithosphere. The expectation is that a sedimentary unit deposited on hot, weak continental lithosphere will load a thin elastic plate to produce a narrow, relatively deep stratigraphic horizon. The same volume of sediment deposited on a cold, thick lithospheric plate will produce a broad, thin horizon. Therefore, stratigraphic cross-sections provide information on the mechanical strength, and by inference the thermal state, of the lithosphere during the period of basin formation.

The thermal state of the lithosphere is often summarized in terms of its thermal thickness L , the depth to which conduction as opposed to convection is the primary mechanism of heat transfer, and its thermal age t , which is the time necessary for the lithosphere to cool conductively to its present state from an initial temperature of about 1,300°C (the temperature maintained at its base). A sedimentary basin is thought to form after horizontal tractions stretch and thin the lithosphere (McKenzie, *D. Earth planet. Sci. Lett.* **40**, 25; 1978). Upwelling of hotter mantle isotherms heats the lithosphere. The subsequent cooling and subsidence of the thinned lithosphere are completely described by the initial thermal age t , the

stretching factor β , and the conductive cooling law for a slab of thickness L . Within the context of this thermal description of the continental lithosphere, Kusznir and Karner quantify the expected variations in mechanical strength of basin lithosphere.

The elastic thickness T_e of very old lithosphere (t more than one billion years) is most sensitive to the thickness of the conductive layer L . If L is 125 km — the generally accepted value for the oceanic lithosphere (Parsons, B. E. and Sclater, J. G. *J. geophys. Res.* **82**, 803; 1977) — then the greatest T_e values observed for old continental lithosphere should be about 50 km. Based on observed values of T_e 3 in excess of 90 km for the old lithosphere beneath the Ganges and Appalachian basins, Kusznir and Karner favour a value for L closer to 225 km for continents.

The stretching factor produces observable variations in the elastic thickness by introducing perturbations to the thermal structure. For the expected range in β , extremely stretched lithosphere will seem to have an elastic thickness more than 10 km thinner than modestly stretched lithosphere for sedimentary loads deposited during the first 10 million years of basin subsidence. Decay of the thermal transient gradually eliminates any β -dependence for loads deposited 100 million years later, so that one must study the deepest layers of sedimentary basins to gain insight into the initiating mechanism.

Other factors complicate the simple relationship between elastic plate thickness and thermal anomalies. For example, all rocks below the depth T_e do not relax instantaneously to relieve the stresses from sedimentary loading. Initially, the

deflection of the continental lithosphere corresponds to $T_e = L$, the full thermal thickness of the lithospheric plate. Relaxation begins at the base of the plate, rapidly at first but then asymptotically approaching T_e with time. Thermally young lithosphere ($t = 10$ million years) essentially reaches its long-term value of $T_e \approx 10$ km after only one million years because of rapid cooling of the plate. In older lithosphere ($t = 300$ million years), stresses at the base of the plate continue to relax, so that the effective elastic thickness of about 50 km decreases by a few kilometres between 1 and 10 million years. Therefore, for young loads less than 1 to 10 million years old, transient effects may cause the lithosphere to appear thicker than predicted by the steady-state theory.

The thickness of the crustal portion of the continental lithosphere is another complicating factor. The rocks in the crust, which are rich in quartz, have a lower activation energy for ductile flow than the olivine-rich rocks in the upper mantle. The lower activation energy causes ductile flow in the lower crust at fairly modest temperatures. For a given temperature structure, continental lithosphere seems weaker as the ratio of crustal to mantle portion increases. Thus two different basins both with thermal structures for 100 million-year-old lithosphere can have T_e values that differ by as much as 30 km if their crustal thicknesses differ by a factor of two.

Provided we can make allowance for the nonthermal parameters affecting the mechanical strength of the lithosphere, the elastic flexural signal preserved in sedimentary basins can be used to determine the thermal history which controls hydrocarbon formation. Kusznir and Karner's work identifies the variables and the magnitudes of the effects involved in such thermo-mechanical studies of sedimentary basins. □

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IMAGE
UNAVAILABLE
FOR
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REASONS

Light photomicrograph ($\times 10$) of *Volvox*. Taken by Ron Oldfield of Macquarie University in Australia, this photograph won first prize in a Polaroid Instant Photomicrography Competition.

Birth defects

Are offspring at risk from their father's exposure to toxins?

from Nigel A. Brown

CONGENITAL malformations can be induced by exposure of the mother to teratogens in early pregnancy, but can they also be caused by treatment of the father before, even long before, conception? The answer, as far as we know it, draws on observations of the effects of genetic lesions, indirectly addressed on page 144 of this issue¹, and on a consideration of potential epigenetic mechanisms.

The various stages of male germ-cell maturation can be differentially susceptible to toxic insult². It is simplest to distinguish two overall phases — pre-meiotic and post-meiotic. The pre-meiotic stem cells produce spermatogonia for the whole reproductive lifespan and they cannot be replaced if destroyed. The lifespans of post-meiotic stages are short by comparison and damaged cells will disappear within one cycle of spermatogenesis, a matter of a few weeks, whereas an abnormal stem cell may be retained for life.

It is possible for treatment of a male to induce genetic damage in germ cells which causes congenital malformation in his offspring. Male mice exposed to X rays or to the mutagen urethane produce fetuses with an increased incidence of malformations^{3,4}. Various common defects are observed, some that would be lethal after birth, others that seem just to be a reduction in fetal size. With post-meiotic exposure of germ cells, up to 4.5 per cent of fetuses are defective, whereas pre-meiotic treatment produces a lower, but still significant, incidence of defects. The malformations are probably caused by both small chromosomal aberrations and gene mutations, and are obviously genetically dominant⁴. Congenital cataracts and skeletal defects have also been observed in offspring sired many months after male mice have been exposed to X rays or ethylnitrosourea⁵⁻⁷. Breeding of these defective offspring has shown (with some technical reservations⁸) that the malformations can be inherited and so are clearly caused by dominant mutations, originally induced in spermatogonia.

Not all embryos produced by mutant spermatozoa result in malformed fetuses. Many embryos die, usually shortly after implantation, due to major aberrations in chromosome numbers or structure induced in post-meiotic stages. Structurally normal offspring can be born with phenotypic changes, ranging from minor coat colour or behavioural abnormalities to potentially lethal biochemical or functional deficits. Limited information suggests that the incidence of cancer in

mice can be increased by paternal treatment with mutagens^{3,9}.

Trasler *et al.*¹ describe birth defects produced by treating male rats with cyclophosphamide, a mutagenic drug widely used in cancer chemotherapy. They confirm that chronic low doses of cyclophosphamide produce many embryonic deaths and report a significant incidence of up to seven per cent malformed fetuses, in contrast to a previous study using a higher dose where only behavioural anomalies in offspring were observed¹⁰. A preliminary report of acute high-dose exposure has also suggested a weak increase in malformations¹¹. The current study uses small groups of treated males, so the number of abnormal fetuses is low and could be due to chance distribution of spontaneous defects, particularly as outbred rats are used. If the malformations are a genuine result of treatment, the observation is significant — many humans are exposed to equivalent doses and there is a suggestion that pre-meiotic germ cells are involved.

Epigenetic mechanisms may also be involved, as some chemicals not usually thought to be genotoxic seem also to affect offspring following paternal treatment. In rodents, paternal exposure to ethanol¹² or narcotics¹³ can lead to reductions in litter size and birthweight, neonatal survival and behavioural deficiencies, but no reproducible increase in malformations. Ethanol may have some genetic effects^{14,15} but it is far from a potent mutagen.

There is little mechanistic evidence to support paternally-induced birth defects by epigenetic events. It seems unlikely that damage to non-genetic components of sperm results in malformations, because recent micromanipulation experiments suggest that sperm cytoplasm is not essential for normal embryogenesis¹⁶. Chemicals can be transported from male to female in seminal fluid or bound to spermatozoa¹⁷ but no fetal malformations have been observed that can be attributed to treatment of gametes at fertilization or of embryos at pre-implantation stages. It seems unlikely that a chemical could retain its activity in the uterus until the embryo reaches the teratogen-sensitive stage of organogenesis.

In humans, epidemiological data suggest that certain occupations increase the risk of fathering an abnormal child¹⁸, but such data are notoriously susceptible to bias from ethnic and socio-economic factors. Two groups of men have been exposed to high levels of genotoxic agents:

survivors of the atom bomb explosions and of cancer therapy. There is no evidence of increased incidence of birth defects in children of people exposed to atomic radiation¹⁹. Men in long-term remission from cancer are less well studied but the current impression is that there is no increased risk of malformation among their children, although this is based on clinical observations with limited power to detect even a modest increase in risk. Because these men represent a unique opportunity to evaluate the possible induction of transmissible genetic damage in humans, efforts should be made to study them carefully.

There has been concern over human exposure to 'Agent Orange' although neither of its components, 2,4-D and 2,4,5-T, or the contaminant dioxin are potent mutagens. No defects have been observed in the offspring of male animals experimentally exposed to dioxin, even though it is an effective teratogen²⁰. Some studies of children of men exposed to Agent Orange have claimed that birth defects are increased but most have not, and none is large or complete enough to be definitive. Several large studies in progress, particularly of Vietnam veterans, should provide more convincing answers²¹.

It would be foolish to advise anything other than extreme caution over the exposure of humans to chemical mutagens when our understanding of the quantitative risks to future generations is so rudimentary. Many fear that the human mutational load is being increased by chemically induced recessive changes which are currently un-noticed. As far as human birth defects are concerned, the limited data are generally comforting but this is not an excuse for complacent inactivity. While we must be thankful that no known chemical induces birth defects by an epigenetic mechanism following paternal exposure, it has to be admitted that such an agent would be an intriguing tool for reproductive toxicologists. □

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Courses and captains

David Dickson

Lost at the Frontier: US Science and Technology Policy Adrift.

By Deborah Shapley and Rustum Roy.

ISI Press: 1985. Pp.223. Hbk \$19.95; pbk \$13.95.

THE way Western countries — and Eastern ones too, for that matter — choose to organize their science usually reflects more general attitudes about the role of the state in the economy. In Britain, for example, central planning of both spheres has always been weak, and the research system is permitted considerable autonomy, provided it observes various loosely-defined social conventions. France, in contrast, applies much stronger centralized control to its science, in line with administrative traditions that stretch back past Napoleon to Louis XIV and his adviser Colbert; while in countries such as Italy, where the state apparatus, though large and cumbersome, has less real power, the organization of science is more pragmatic and reflects initiatives taken by influential individuals.

The United States is no exception to this pattern. Largely as a result of the Congressional system, the structure of federal support for science — like that for the broader economy — results as much from efforts to balance pressures from a mosaic of frequently-conflicting lobby groups as it does from any carefully-crafted philosophy or government strategy. The evolution of scientific institutions in the United States provides a concrete testimony to the effectiveness of these pressures, whether in the form of a new research institute added to the National Institutes of Health at the prompting of a group of medical charities, or a space station eagerly promoted by sectors of the aerospace industry.

One result is that it is virtually impossible to identify a single, clear science and technology policy in the United States. Is this the most sensible way of running a research budget that is larger than the economic turnover of some European countries? Deborah Shapley, previously Washington correspondent for *Nature*, and Rustum Roy, head of the materials science laboratory at Pennsylvania State University, say no. The argument sketched out in their book *Lost at the Frontier* is that, whatever its undoubted strengths, the American research system is not contributing as much as it could be to the country's well-being, largely because it is insufficiently co-ordinated.

The book starts from the thesis that the bold plans put forward by Vannevar Bush in the years immediately following the Second World War for the renovation of American science have been betrayed by a lack of imagination and leadership. Bush

argued for increased and continuous government support for basic science — “the endless frontier”, to quote the title of his famous 1945 report — primarily as a means of achieving economic prosperity; yet this objective rapidly gave way, argue Shapley and Roy, to the philosophy that basic science was a desirable end in itself.

This “basic-research-is-best” ideology, they say, still dominates the thinking of both the scientific community and the Reagan administration. Not only has it contributed to a downgrading of research explicitly aimed at practical goals, but it has also led research workers to consider that they have a right to continued and generous support from government, regardless of whether their results are of use or value to anyone else. And this in turn has driven a wedge between the world of science and that of its technological applications which can be blamed for many of the problems currently faced by the American economy.

Even if much of what Shapley and Roy have to say about such problems has been heard many times before, their criticisms are worth repeating. It is still too easy for scientists to hide behind the argument that, since their sole motivation is intellectual, they are under no obligation either to discuss the social implications of what they are doing, or indeed to concern themselves in any way with its potential technological applications. It is also true that there are some areas of research where a greater consistency in federal support would have been more productive. One of the book's most revealing case studies concerns Roy's own field of ceramics, where wild fluctuations in government enthusiasm — and thus funding — have clearly been harmful when compared with the sustained interest that the field has received in other countries, particularly Japan.

Three central questions, however, are raised by the general thrust of Shapley and Roy's argument. Is it true that American research policy remains dominated by a “basic-research-is-best” attitude which undermines efforts to link science and technology? Would the situation be improved by a central, guiding authority? And could such an authority operate effectively within the existing institutional structure of American politics?

In each case, there is firm evidence to contradict the claims made by Shapley and Roy. On the first, for example, it is certainly true that, through a process

which started during the Carter administration and has accelerated under President Reagan, the main emphasis in government funding for science has swung back from “applied” research programmes in fields such as energy and transport towards more “basic” projects. Yet as government spokesmen frequently emphasize, this shift has taken place not for abstract, cultural reasons, but because of the twin beliefs, now widely shared in top government and industrial circles, that basic research represents a long-term investment in the technology of the future, and that the market-place — rather than a political planning committee — is the appropriate mechanism by which this investment should be realized.

Secondly, attempts to impose directions on science from the centre can be stifling rather than stimulating, however enlightened the policy-makers. Shapley and Roy describe at some length the successful efforts of Japan; but they make no mention of France, where the experience of central planning for science has been much less fruitful. Nor do they look closely at the highly decentralized (and basic-research-orientated) structure of the biomedical research community in the United States, whose vitality, when combined with the vigour and enthusiasm of the venture capital market, has been the key factor in determining America's dominant position in the global biotechnology industry.

Finally, their proposals are put forward in a political vacuum, assuming that rational discussion will lead to consensus between experts from industries, universities and government on appropriate national research priorities. In practice such priorities (ranging from synthetic fuels to the Strategic Defense Initiative) are frequently determined by non-technical arguments. The fact may drive some scientists to despair; but it needs to be seriously considered by anyone who proposes radical changes in the current pattern of decision-making. For example, in discussing the increasing restrictions placed on scientists attending conferences on topics that could have military implications, the authors describe this as merely the result of misguided political interference in the practice of science. They do not analyse in any detail the various factors that have given rise to these conflicts, such as the increasing amount of “basic” research that has a clear and direct relevance to both civilian and military technology almost simultaneously.

Shapley and Roy make several interesting suggestions about how the organization of American science could be improved. These range from various proposals for streamlining the peer review system (for example, by increasing the number of multi-annual grants and placing greater weight on prior performance), or imposing a greater concentration of government

funds on centres of research excellence, to allowing scientists to spend part of their working time doing voluntary teaching in schools (a practice recently adopted in France). Other suggestions are included in a series of responses to their case solicited from various prominent figures such as President Eisenhower's science adviser, James R. Killian, and Edwin H. Land of Polaroid camera fame. (Unfortunately most of these luminaries use the opportunity to present their own views on how science should or should not be run, rather than give detailed opinions on the ideas of the two principal authors.)

In the end, however, any arguments about the optimal strategy for science and technology which emphasize questions of internal structure, such as the relative weighting to be given to basic, "strategic" and applied research, without paying equal attention to the political logic of the external pressures which frequently determine this internal structure, inevitably appear idealistic, if not utopian. To argue, like Shapley and Roy, that American science is "lost at the frontier" and that science policy is "adrift", implies that a map and a rudder would answer the basic problems. But this raises the all-important question of how (or whether) agreement will be secured on a single map — or whose hand will be allowed on the rudder. And it can be argued that one of the most important changes in American science policy over the past decade has been the increasing influence that private industry and the military have been able to exert over precisely these processes.

There has been a long tradition of members of the scientific community declaring a strong aversion to the untidiness of the procedures by which rudimentary political issues, including the control of the direction of science and technology, tend to be resolved in society. If only, it is often argued, the organization of science — and of society too — could be made more scientific, conflicts and inefficiencies would be eliminated and the whole enterprise would immediately become more productive.

Shapley and Roy clearly share such hopes. Their optimism is to be admired, and there are sectors of the research enterprise in the United States where their solutions are certainly appropriate. But their insistence on the supremacy of scientific management risks overlooking the extent to which the cracks currently appearing in the American research system can be read as symptoms — rather than causes — of broader economic and political conflicts. It also underestimates how far these latter factors need to be taken into account if a truly rational policy for science and technology is to be achieved. □

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Truths of planetary evolution

S.R. Taylor

The Geology of the Terrestrial Planets. By Michael H. Carr, R. S. Saunders, R. G. Strom and D. E. Wilhelms. NASA: 1984. Pp. 317. \$16 (North America), \$20 (elsewhere).

EVERY geologist should be familiar with the landscapes and geological processes of the Moon and nearby planets, if not on account of their inherent interest then because of the insight and perspective which they shed on terrestrial geology. The authors of this volume have all made signal contributions to planetary geology, clearing away in the process much of the pre-Apollo confusion, and here provide an up-to-date and readable summary of our present understanding of the subject. Those scientists who dismiss geological evidence as merely pictorial in nature would do well to read this book and perhaps discover that hard truths about planetary evolution do emerge from the study of landscapes. Even students of cosmology should be interested to learn that the radius of Mercury shrank by a kilometre or so about 4×10^9 yr ago but, like the Moon, has remained static since that time, placing limitations on the variation of G and causing disarray among proponents of expanding Earth hypotheses.

Many questions remain to be answered. Are the smooth plains of Mercury really analogous to lunar maria or are they ejecta blankets? The composition of Mercury is clearly distinct from that of the Moon, and the recent failure to confirm, by reflectance spectra, the presence of Fe^{2+} on the surface must arouse doubts about their volcanic nature. A further problem is whether heavily cratered surfaces, on Mercury and in the lunar highlands especially, are saturated with craters, or constitute a production population. In the latter case, we can read the history of the surface since accretion. The former possibility, that we see only the record of the closing stages of the great meteoritic bombardment, implies that the observed lunar highland surface is no older than 4.1 or 4.2×10^9 yr. This seems more likely to me. Equally contentious is the existence of the giant Procellarum basin, 3,200 km in diameter, which may underlie much of the visible face of the Moon. Wilhelms's overview of lunar multi-ring basin stratigraphy removes the basis for the once popular "lunar cataclysm" hypothesis. The nature of the Tharsis bulge on Mars, whether a plateau uplifted by internal forces, or constructed by volcanic flows, remains open, but evidence for the former presence of flowing water on Mars seems unequivocal. The surface of Venus is perhaps analogous to

earliest Archaean times on the Earth. Evidence of granitic or other siliceous rocks, so typical of the continental crust of the Earth, appears to be minimal for Venus and the other planets; such rock types, familiar even to city dwellers as facings of buildings, may thus be uniquely terrestrial, a consequence of the abundance of liquid water.

The geological study of the terrestrial planets continues to flourish. More recent discoveries of many multi-ring basins on Mercury and the possibility that the lunar megaregolith is 25 km deep do not appear in the book, but it is nonetheless commendably up to date. Overall editorial control appears to have been satisfactorily light, so that the expertise and style of the individual authors (Strom on Mercury, Saunders and Carr on Venus, Carr on the Earth and Mars, and Wilhelms on the Moon) show through clearly.

Minor failings include an inadequate index of three pages (partly offset by a four-page table of contents). References are given at the end of the book, by chapter, but a combined reference list would have been preferable. Don Wilhelms will be especially chagrined to find that his beautiful plates (C and D on pp. 202–205) of the palaeogeography of the Moon have been transposed. And the maps, 18 pages of them at the end of the book, are really too small; NASA, unconstrained by the limitations of commercial publishers, would have been better advised to have included their useful larger series of planetary maps in a pocket.

This is a valuable contribution to the literature dealing with the terrestrial planets. It provides an excellent basis for university courses in the geology of the terrestrial planets as well as solid reading for geologists involved in the subject. □

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Chemical reference in English

Two of the oldest and best known reference works for chemists, *Beilstein Handbook of Organic Chemistry* and *Ullmann's Encyclopedia of Industrial Chemistry*, published in German for 104 years and 71 years, respectively, are now appearing in English.

Beilstein consists of a main series and five supplementary series. Publication in English has started with Vols 17–27 (heterocycles) of the fifth supplementary series (EV) of the fourth edition, covering the literature from 1960 to 1979. Executive editor of the work as a whole is R. Luckenbach of the Beilstein Institut, publisher is Springer-Verlag.

Ullmann's in English is to be a 36-volume series — 28 "A" volumes (alphabetically arranged articles) and 8 "B" volumes (basic knowledge), to appear at the rate of three or four a year — and commenced with Vol. A1 of the fifth edition, *Abrasives to Aluminum Oxide*. Executive editor is W. Gerhartz and publisher is VCH.

It came from space

Peter Kemp

Comet Halley: A Novel. By Fred Hoyle. Michael Joseph: 1985. Pp. 410. £9.95. To be published in the United States later this year by St Martin's Press.

WHAT'S most astronomical about Fred Hoyle's new novel, *Comet Halley*, is the number of different subjects packed into its pages. The book sets out to be a love-story and a political satire, a thriller, a spy mystery, an exercise in science fiction, a pacifist tract, a comedy of Cambridge manners and an essay in evolutionary theory. Like the heavenly body at the heart of its narrative, it whizzes along — sometimes garish, sometimes gaseous — throwing off material in all directions.

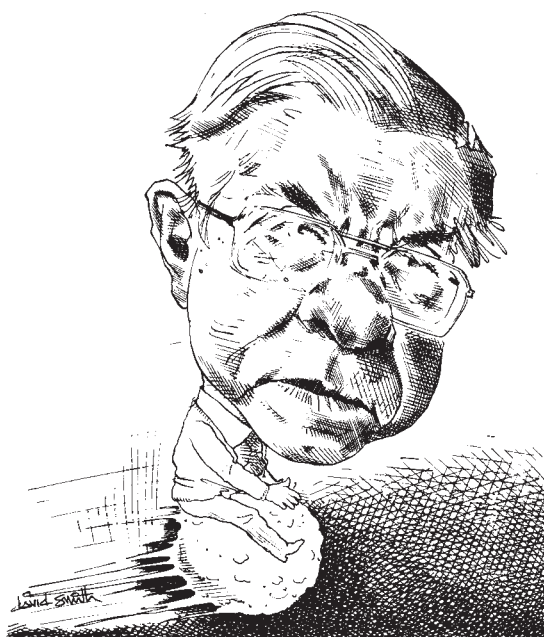
The story opens with scientists in Cambridge, England, amazedly receiving radio messages beamed from Comet Halley. As excitement about this mounts, so does the local death-rate. Researchers expire in unusual circumstances; there are murders, lethal booby-traps, a deadly bombing; several characters are found mysteriously charred to death. Gradually sorting out what's behind all this is the improbably-named Isaac Newton: no relation to the seventeenth-century luminary but like him in opening up a new dimension in thinking. For, along with Frances Margaret, a brainy beauty who becomes his lover, he reaches the conclusion that Comet Halley harbours life — and life of a civilized and civilizing disposition. Comprising "a biologically generated nuclear reactor", it seems, the comet is directing itself to the prevention of nuclear war on Earth.

Soon, in his efforts to assist the comet in its pacific purposes, Newton is striding down the corridors of power (echoes of C. P. Snow keep reverberating through this ambitious sprawl of a novel), trying to convince politicians that humanity is on the brink of a momentous breakthrough. If only nations would realize the folly of pouring money into military budgets and divert resources into the building of telescopes instead, it's argued, then they will be able to receive and re-direct messages from Halley and a host of other would-be communicative comets throughout the Galaxy. The Earth, Newton points out, could become the hub of "a sort of cosmic telephone exchange", "an extra-terrestrial communications system". Since each comet is "a giant brain by our standards", the linking of them all into one vast network of brainwaves must result in hitherto undreamed-of intellectual advances.

Factors standing in the way of this — scepticism, militaristic bigotry, the stockpiling of nuclear weapons — are robustly geyed and decried in this book. Likewise, those who attempt to deflect Comet

Halley's benign aims find themselves having a hard time. When not actually reduced to blackened stalagmites by long-distance emissions from the peace-loving projectile, they succumb to curious maladies: in Moscow, the Politburo go down with "the mad itch", an extra-terrestrial ailment that plagues people into frenzies of nude scratching; the population of Washington is afflicted by a strange derangement which brings on an irresistible urge to hibernate. Finally, ramming its aims home with some emphasis, the comet bombards the Arctic with a guided missile, pulverizing the ice-cap and hastening a change in the climate of opinion on Earth by drastically altering the planet's weather.

As if this weren't striking enough, Hoyle throws in further excitements for good measure: there's a particularly grisly sub-plot involving "a flying torture chamber" staffed by a quartet of odd-shaped psychopaths: a sadist with a Neanderthal skull and larger teeth than is customary, an ape-like giant, a vicious dwarf and a "ghastly waxen-faced creature" with shoulder-length white hair who wields a syringe filled with some unbearably agonizing concoction. Injecting one dramatic incident after another into his plot, Hoyle is generous to



a fault with dramatic incident — but always for didactic purposes. In as eye-catching a way as possible, *Comet Halley* is designed to trail its author's views on the emergence of life — and the likely extinction of it on Earth unless there's a change in thinking. □

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Grains of evidence

Peter D. Moore

The Palynology of Archaeological Sites. By Geoffrey W. Dimbleby. Academic: 1985. Pp. 176. \$45, £34.50.

POLLEN analysis has proved a valuable asset in the investigation of archaeological sites, though the problems involved in its application have not always been adequately appreciated. Only rarely are such sites conveniently located close to deep lake or peat sediments which permit extensive environmental reconstruction; more often the archaeologist has to make do with a buried soil, an infilled ditch or a compacted cave deposit. And such unconventional sources require specialized techniques of sampling and interpretation.

Geoffrey Dimbleby has been the pioneer in this field and has now brought together the collated wisdom of his years of research. It is a book with a strong practical emphasis, reflecting the fact that Dimbleby has been concerned not only with the laboratory analysis of samples but also with the collection of materials in the field. Such field experience shows through in the discussion of the various sources of polleniferous material and in the helpful warnings given about the limitations

that are imposed by different site factors.

Very naturally, much of the book is given over to soil pollen, Dimbleby's principal interest. This subject, which has been neglected and undervalued by most palynologists, has long required the kind of detailed and frank scrutiny which Dimbleby has here provided. He examines the many processes, such as the downwash and the faunal mixing of pollen, which prevent the simple interpretation of soil pollen profiles as stratified sequences.

As well as dealing with interpretive problems, the book contains many of Dimbleby's soil pollen diagrams which illustrate the kind of information to be gleaned from soil pollen profiles. Perhaps this aspect should have had less prominence, however, since these diagrams are readily available elsewhere and undoubtedly contributed to the book's high cost.

The author's cautious attitude to the application of pollen analysis and interpretation of the results may dismay some archaeologists who expect rather too much from the technique. But the book should be welcomed as a timely damper on those exuberant spirits whose enthusiasm outpaces their data. □

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Going through the changes

Mark Johnson

On the Nature of Human Plasticity. By Richard M. Lerner. *Cambridge University Press: 1984. Pp.208. £17.50, \$29.95.*

"PLASTICITY" is one of those labels that has taken on a variety of different, but overlapping, meanings. For example, the neurophysiologist may use the term to refer to the capacity of the nervous system to regenerate or reorganize after injury or environmental change, while some psychologists choose to reserve the term for the possible range of variation that can occur in individual development. Because of this confusion of meanings, it is essential that anyone setting out to integrate information on "plasticity" from various disciplines (in this case genetics, neurobiology, evolution, individual and group psychology) should adopt a clear and precise definition. In his book, however, Richard Lerner appears to be content to allow his interpretation of the word to emerge from the interaction between the reader and the 174 pages of text. This is both a strength and a weakness. While it enables the author to apply the term to everything from molecular genetics to social relationships, the price to be paid is the rather doubtful usefulness of his conception within some levels of explanation.

Two main theses with regard to human development are put forward in the book. First, Lerner suggests that "plasticity" is not confined to the early years but exists throughout the entire life-span. The "life-span" developmental view is a recent school of thought in psychology which emphasizes the capacity for change in the adult. This emphasis on the adult allows its proponents, including Lerner, to use "plasticity" to refer to any changes in structure or function in the organism. However, when this conception of the term is confused with "plasticity" as a specific response to the environment, which it is in the book, the reader is left with the implication that all developmental change is environmentally specified. And this while claiming to have moved beyond the old nature-nurture debate.

The second main thesis is that variables and processes at different levels (biological, individual psychological, dyadic, social and so on) may contribute to human functioning at any one time. These levels do not work independently, in parallel, but are reciprocally influential. Each level is seen to be "embedded" within the others. Lerner formalizes this idea in a framework for describing the development of individuals within their multi-levelled environment. However, as he himself admits, this framework merely constitutes a specification of the domain

within which future theory and research about individual development should be generated. Its usefulness can only be assessed by the success or otherwise of the models it gives rise to. Even if successful at the psychological level, it is not clear whether such a framework would be appropriate at, for example, the neurobiological level. Equivalence of terminology does not imply equivalence of mechanism.

Lerner sees the ideas presented in the book as being of consequence for the clinician. Since each "level" is engaged in dynamic interaction with the others in which it is embedded, it follows that intervention by the clinician at one level will have effects at other levels. For example, changing an individual's diet may have an influence on neurotransmitters which may then have effects on behaviour, and

thence on dyadic and other social relationships. The effects at other levels may be beneficial or detrimental, but in order to predict them some understanding of the mechanisms of interaction between levels is required. Lerner offers us only description, and description is not adequate for prediction.

In his foreword, Paul Baltes states that despite his approval of the book he is convinced that the author will be criticized for his commitment to interdisciplinary breadth. On the contrary, such a commitment is to be applauded. However, the synthesis we are offered in this book is, like all mirages, attractive at a distance but elusive close up. □

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Nonsenses of today

Joseph Silk

Perfect Symmetry: The Search for the Beginning of Time. By Heinz R. Pagels. *Simon & Schuster: 1985. Pp. 329. \$18.95. To be published in Britain later this year by Michael Joseph.*

PARTICLE physics and cosmology have developed a symbiotic relationship. While it would take a particle accelerator stretching from here to Alpha Centauri to smash particles together at energies approaching the grand unification scale (and we may safely consider this to be outside the future budgets of CERN or Fermilab), such energies were reached routinely in the very early Universe. Hence the attention being paid by particle theorists to constraints from cosmology. Already, many exotic theories have been eliminated, and others, phoenix-like, have arisen. Does the dark matter that pervades the visible Universe consist of massive chunks of quark matter at nuclear density? Or does it consist of shadow matter, a whole universe of stars and galaxies, invisible to us apart from their gravitational interaction?

This book by Heinz Pagels, a particle physicist, provides a lucid introduction to the interplay between cosmology and particle physics. He describes the motivation and basis for grand unification theories, and their role in cosmology, and includes a chapter on the inflation of the Universe, one of the most notable results of the synthesis between particle physics and cosmology. Inflationary cosmology provides a scientific basis for understanding why the Universe is so large, and why it is so isotropic (that is, similar in all directions). It explains why the Universe is almost, but not quite, uniform: from the primordial fluctuations in density, the galaxies eventually grew.

Although the hottest topics from last year's conference season, such as quark nuggets or shadow matter, are inevitably absent, there is a sufficiently solid introduction to the new physics that the reader will have little difficulty in making sense of the latest developments that appear in the semi-popular press. But to give him full credit, Pagels does not hesitate to tackle some vertiginous issues. What happened before the big bang? What was the origin of everything? Our Universe may well have begun as a quantum fluctuation: before, there was nothing, not even time or space, but absolutely nothing.

Much of the text is devoted to an account of the astronomer's Universe. The latest discoveries from space astronomy are described, ranging from the infrared-emitting embryo stars being hatched deep within dense interstellar gas clouds to the tenuous hot plasma glowing in X-rays throughout megaparsecs of intergalactic space in the great galaxy clusters. All of this, and a capsule description of the evolution of the big bang, from the beginning of the knowable Universe, some 10^{-43} s after the initial singularity, until about 1×10^{10} yr later.

The book is written for the intelligent layman, and thus non-technical and not unduly laden with jargon. It is, however, replete with quotations that help communicate the excitement at the frontier of science. My favourite is Eddington's reaction to the discovery of the first white dwarf star, Sirius B, supposedly made of material thousands of times denser than any known matter: "Shut up. Don't talk nonsense". Eddington's comment has been repeated many times since in reaction to novel observations or experimental data: seventy years later, it remains to be seen whether we have truly learned our lesson. □

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Lorentz resonances and the structure of the jovian ring

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Charged dust orbiting through spatially periodic planetary magnetic fields will experience time-variable electromagnetic forces. When the forcing frequencies are nearly commensurate with the particle's orbital frequency, the particle undergoes large out-of-plane and radial excursions. Specific 'Lorentz' resonances, corresponding to particular spatial periodicities in the magnetic field, occur on either side of synchronous orbit. We describe here Lorentz resonance locations and strengths for the jovian and saturnian rings. The boundaries of the halo of the jovian ring, and perhaps other ring structure, are near resonances.

ORBITAL resonances, those positions at which periodicities in perturbations are commensurate with the orbital period of a test particle, have long been suspected to govern much of the structure of the Solar System. For example, gaps and concentrations are found in the asteroid belt at positions where the orbital period of a particle would be close to a simple fraction of Jupiter's orbital period^{1,2}. Similarly, the Voyager spacecraft discovered some 50 cases of ring edges, isolated narrow ringlets, gaps or wave trains in Saturn's rings located at resonances with nearby satellites^{3,4}. On the other hand, it is unlikely that the uranian rings reside at three-body resonances (as once suspected⁵) because the putative resonances are too weak^{6,7}. As well as the above resonances, which are produced by the periodic pulls of orbiting perturbers, 'lumpiness' in a planetary gravitational field could affect particles having orbital periods that are fractions of the planet's spin period⁸ (compare with ref. 9); but recent refinements in ring dimensions show no unusual features at such locations³.

Only in the case of Jupiter's ring¹⁰ have resonances not been invoked extensively (see ref. 11), perhaps because satellite perturbations are weak since neighbouring moons are either distant or small, and because collective gravitational effects are thought to be non-existent in such a tenuous structure¹⁰. We show here that several features of the jovian rings are, however, near 'Lorentz' resonances and we outline how this connection might be enforced^{10,12}. We also point out that the ring's outer edge is close to a feeble satellite resonance; if this association is confirmed by the Galileo spacecraft, the workings of resonances may have to be reappraised.

Lorentz resonances

Electromagnetic forces have been shown to be significant for jovian ring particles, especially as the possible cause for out-of-plane motion^{10,12-15}. Accelerations $[(q/mc)\mathbf{v} \times \mathbf{B}]$ resulting from Lorentz forces are fairly strong perturbations because: (1) jovian particles have large velocities $\mathbf{v}$ across an intense magnetic field $\mathbf{B}$; and (2) their electric charge-to-mass ratios q/m are generally larger than for members of other ring systems because most visible jovian grains are tiny (of the order of a micrometre^{10,12}) and adopt an electric potential V determined by the surrounding magnetospheric plasma and estimated at -10 V (refs 10, 16, 17). The jovian magnetic field is far from an aligned dipole^{18,19}; in a multipole representation, the quadrupole and octupole terms contain 30% of the strength¹⁸. Accordingly, the Lorentz force on a jovian ring particle varies substantially even for a particle of fixed charge travelling along an equatorial circular orbit. Specifically, a particle in a pure dipole field would experience varying radial forces if the dipole were tilted or were offset equatorially; a constant latitudinal force would be produced by an axially offset field and a variable one by a tilted field.

A particle orbiting a planet with a spatially periodic magnetic field will experience a temporally-variable electromagnetic force

as it passes through the various frequency components of the field. Lorentz resonances are said to occur at those positions where orbital periodicities match a periodic component of the Lorentz force, for then the perturbing force will be in phase with the particle's natural oscillatory motion. This elementary viewpoint has been supported by a perturbation solution¹⁵, where the equations of motion are found to be those of forced, linear harmonic oscillators. To locate Lorentz resonances, we assume that in the neighbourhood of the ring the jovian magnetic field $\mathbf{B}$ can be derived from a potential function ψ (refs 18, 19) due either to current sources in the planet or at great distances from it. ψ can be represented by an infinite series of spherical harmonic functions, such that for internal sources

$$\mathbf{B} = -R\nabla \sum_{l=1}^{\infty} (R/r)^{l+1} \sum_{k=0}^l P_l^k(\cos \theta) [g_l^k \cos k\phi + h_l^k \sin k\phi] \quad (1)$$

where R is the planet's equatorial radius, (r, θ, ϕ) are the usual spherical polar coordinates measured relative to the rotating planet, $P_l^k(\cos \theta)$ are the Schmidt-normalized associated Legendre functions, and g_l^k, h_l^k are the observationally-determined Schmidt coefficients defining the field.

As each component of the field is a trigonometrical function of the longitude ϕ , an equatorial particle moving with a mean orbital angular velocity n would experience the k th spatial component of the field to vary with a frequency $k(n - \Omega)$, where Ω is the spin rate of the magnetic field (planet). Lorentz resonances are present at those radial positions where this frequency matches local epicyclic frequencies, defined as the rates κ and μ at which a test particle displaced by an arbitrary small amount will oscillate about the reference circular orbit in the horizontal or vertical directions²⁰. Ignoring planetary oblateness for the moment, the horizontal (κ) and vertical (μ) epicyclic rates equal n . Hence the resonance condition is approximately

$$k(n_k - \Omega) = n_k \quad (2)$$

where n_k is the mean motion of a particle at the k th resonance; a positive k is appropriate if $n_k > \Omega$ (that is, resonances interior to synchronous orbit defined as the radius R_{syn} where $n = \Omega$), whereas a negative k corresponds to exterior resonances. Morfill and Grün²¹ have found independently a similar resonance in connection with the motion of charged dust across the sector structure of the interplanetary magnetic field.

Figure 1 illustrates the operation of a typical Lorentz resonance pair. The radial portion of the $k=2$ magnetic field component is outward in two quadrants and inward for the remaining two, if we define zero longitude such that $g_2^2 \equiv 0$ in equation (1); although the strength of the $k=2$ component varies sinusoidally across a quadrant, that is unimportant for this heuristic description. Negatively charged particles at the interior and exterior resonant orbits each experience out-of-plane Lorentz forces. The force on the interior particle at the location

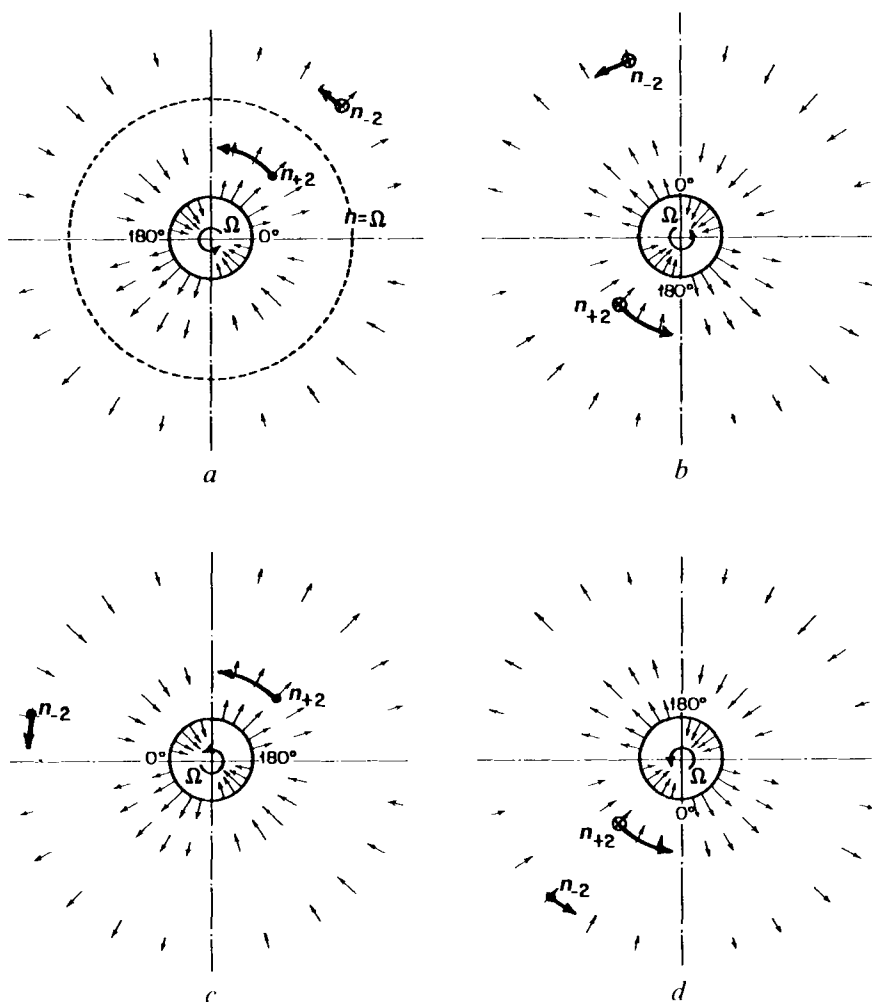


Fig. 1 Two equatorial particles—one at the interior $k = +2$ Lorentz resonance and another at the exterior $k = -2$ resonance—in four views from the North Pole. The outward and inward arrows represent the radial part of the planet's magnetic field that has $k = 2$ periodicity; the planet, drawn as a solid circle, rotates by $\pi/2$ (relative to inertial space) in each successive panel. For each of the two orbits the Lorentz forces ($\bullet$, out of the plane of the paper and $\oplus$, into the plane of the paper) change sign every π radians of inertial space; these forces thereby induce north-south motions, that is, they produce inclined orbits. Large radial excursions of the orbit will be similarly generated because the latitudinal field also contains a $k = 2$ periodicity. The synchronous orbit ($\Omega = n$) is shown dotted in a.

shown in Fig. 1a is out of the plane of the paper (as v , the velocity relative to the field, is positive) but the force on the exterior particle at the same longitude is into the plane of the paper (as relative v is then negative).

In Fig. 1b, the magnetic field has rotated by $\pi/2$ and the interior particle is back to its starting point (but now above the opposite face of the planet). The force on the particle at the $k = -2$ resonance has finally reversed, so both are subject to southward forces.

After another quarter rotation of the planet (Fig. 1c), the inner particle is back to its starting point (but now above the opposite face of the planet). The force on the particle at the $k = -2$ resonance has finally reversed, so both are subject to southward forces.

In Fig. 1d, the two particles are aligned once again, exactly π from their configuration in Fig. 1a, and both experience forces with opposite sign from one another and from those in Fig. 1a. The cycle now repeats itself. During its 3π longitudinal excursion, the interior particle has been pushed up and down twice, whereas the particle at the exterior resonance has only travelled π radians and gone through half a force cycle. Each has been pushed up on one side of its orbit and down on the opposite side exactly in phase with the orbital motion.

In terms of equation (2), the interior resonance of Fig. 1 is found at that radius where the mean motion satisfied $2(n_{+2} - \Omega) = n_{+2}$, or $n_{+2} = 2\Omega$, whereas the exterior resonance is defined by $-2(n_{-2} - \Omega) = n_{-2}$, or $n_{-2} = (2/3)\Omega$; as already shown in Fig. 1, $n_{+2}/n_{-2} = 3$. Clearly, the $k = +1$ (interior) resonances can never be achieved because the perturbation frequency observed by the particle ($n - \Omega$) is everywhere $< n$. Furthermore, resonances become more and more closely spaced (but generally also

weaker as we will show) when the synchronous orbit position is approached from either side.

Using Kepler's third law to express n in terms of r , the k th resonance is approximately located at

$$r_k = \left[\frac{k-1}{k} \right]^{2/3} R_{\text{syn}} \quad (3)$$

The interior k th resonance is found by substituting positive integers while the exterior k th resonance is located with negative integers.

R_{syn} can be calculated to be at $2.2444R_{\text{J}}$ for Jupiter, while for Saturn $R_{\text{syn}} = 1.8651R_{\text{S}}$, if the effects of the higher-order gravitational moments J_2 and J_4 are included.

To locate the resonances more precisely, the expressions for the epicyclic frequencies κ and μ should include higher-order gravitational moments, which cause $\kappa < n < \mu$ and, therefore, mean that the vertical and horizontal resonance positions split. Substituting classical expressions²² for n , μ and κ , accurate to J_2 , into equation (2) with the right-hand side replaced by μ , the k th vertical Lorentz resonances are at

$$r_{kv} = [(1 - k^{-1}) + (3/4)(1 - 3k^{-1})J_2(R/r_k)^2]^{2/3} \times R_{\text{syn}}/[1 + (3/4)J_2(R/R_{\text{syn}})^2]^{2/3} \quad (4)$$

Horizontal Lorentz resonances are found by replacing n on the right-hand side of equation (2) by κ to yield

$$r_{kh} = [(1 - k^{-1}) + (3/4)(1 + k^{-1})J_2(R/r_k)^2]^{2/3} \times R_{\text{syn}}/[1 + (3/4)J_2(R/R_{\text{syn}})^2]^{2/3} \quad (5)$$

(Note that the signs of the second and fourth terms for κ are wrong in ref. 22.) Expressions (4) and (5) are equally valid for

interior and exterior resonances as long as k is taken to have a positive and negative sign, respectively. As $J_2 \approx 0(10^{-2})$ for all planets, these expressions can be solved rapidly by successive approximation. More exact expressions for n , κ and μ involve J_4 and J_2 (ref. 2), which introduce corrections of $0(10^{-4}-10^{-5})$ to equations (4) and (5). These terms have not been included because comparable or larger corrections will arise by direct electromagnetic effects: n itself will depend on the particle's charge-to-mass ratio because centrifugal balance should include the constant radial component of the Lorentz force, which generally will shift resonances by small amounts that depend on k .

To a lesser extent, the exact positions of the resonances are uncertain because the various harmonics of the magnetic field might rotate at slightly different rates (D. J. Stevenson, personal communication); for example, the non-dipole part of the Earth's field drifts westward relative to the surface at $\Delta\Omega/\Omega \sim 10^{-5}$, and, accordingly, resonant positions would be translocated by a comparable fraction.

We estimate the relative strength of the k th Lorentz resonance from the latitudinal force that has k periodicity. This is proportional to $v_{\phi k} B_{rk}$, where $v_{\phi k} = r_k(n_k - \Omega)$ is the longitudinal speed of the particle across the part of the radial magnetic field B_r that has k periodicity. We estimate B_{rk} by the term in equation (1) that has k periodicity and the lowest power of R/r_k . The coefficient of this term is $(|k|+1)(R/r)^{|k|+2} P_k^k(0) C_k$, where C_k is defined as $[(g_k^2 + (h_k^2)^2)]^{1/2}$ and serves as a measure of the relative importance of the k th component of the field. Hence the strength S_k of the k th resonance is proportional to

$$S_k \sim \frac{(|k|+1)}{(1-k)} \Omega R (R/r)^{|k|+1} P_k^k(0) C_k \quad (6)$$

This expression pertains to latitudinal forces; the radial forces are comparable.

The resonances that we have just described are appropriate for the linearized problem. A more complete treatment would incorporate nonlinear terms, including a coupling between the radial and latitudinal motions. Based on the classical theory of coupled, forced nonlinear oscillators, we anticipate that the solution to the full nonlinear problem will contain features not present in the strictly linearized equations. The locations of the higher-order (nonlinear) horizontal Lorentz resonances can be found by generalizing equation (2):

$$k(n_k - \Omega) = K n_k \quad (7)$$

where K is a small integer; for vertical Lorentz resonances, the right-hand side of equation (7) would also be $K n_k$ with the appropriate higher-order corrections included in n_k . Because of non-linearity, super- and sub-harmonic resonance motions might be achieved at such positions. New locations are introduced through equation (7) and the previous positions of equation (4) can be shifted slightly because the 4:2 resonance does not lie on top of the 2:1 resonance since higher-order corrections enter them differently.

Resonance locations and strengths

The locations of the low-order Lorentz resonances for Jupiter and Saturn according to equations (4) and (5) are given in Table 1, where we have taken $J_2 = 0.01473$, $J_4 = -0.00059$ and $R_{21} = 71398$ km for Jupiter²³, and $J_2 = 0.01630$, $J_4 = -0.00092$ and $R_n = 60,330$ km for Saturn²⁴; J_4 is used only in computing R_{syn} but not in equations (4) and (5). In agreement with our earlier statement, we see that a vertical $k = +1$ resonance is not permitted; the horizontal $k = +1$ resonance has a solution that will lie within the surface of the planet unless the planet is unreasonably oblate and dense (J. J. Lissauer, personal communication).

Relative strengths for the various k resonances in the jovian and saturnian ring systems are also listed in Table 1. To represent the planetary magnetic fields we have used the Pioneer 10 (3I, 2E) model¹⁹ for Jupiter since that spacecraft traversed a nearly equatorial orbit in the region of interest, and the JGR80 model for Saturn^{25,26} as it is the only representation involving

Table 1 Locations and relative strengths of Lorentz resonances

Resonance order k	Jupiter ($1R_{21} = 71,398$ km)		Saturn ($1R_n = 60,330$ km)	
	Location r_k/R_{21}	Relative strength*	Location r_k/R_n	Relative strength*
interior				
+1	—	—	—	—
+2	V 1.4065 H 1.4276	4,004	1.1653 1.1930	155
+3	V 1.7102 H 1.7190	629	1.4200 1.4315	22
+4	V 1.8513 H 1.8565	$\sim 200^\dagger$	1.5378 1.5447	$\sim 10^\dagger$
R_{syn}	2.2444		1.8651	
exterior				
-4	V 2.6046 H 2.6023	$\sim 20^\dagger$	2.1648 2.1615	$\sim 1^\dagger$
-3	V 2.7188 H 2.7163	49	2.2596 2.2560	2
-2	V 2.9408 H 2.9375	145	2.4438 2.4394	6
-1	V 3.5618 H 3.5575	574	2.9592 2.9537	3

* Based on equation (6), using r_k as given here and magnetic field coefficients estimated from refs 19, 26. The tabulated values are 10^4 times those computed from equation (6); so as to have non-dimensional results that can be compared, we have not included ΩR in equation (6) for Jupiter and have divided the Saturn result by ΩR of Jupiter.

† The appropriate magnetic field coefficients are unknown and are arbitrarily selected to be similar to the planet's octupole terms.

$k = 3$ sectorial harmonics. The particular values for the spherical harmonic coefficients are not especially accurate, especially for the higher-order terms; in fact, the fields probably change over long times as does the Earth's. Nevertheless, we emphasize that although resonance *strengths* will vary, depending on the precise representation selected for the magnetic field (see refs. 18, 19), the resonance *locations* rely merely on the spatial periodicity of the field, which depends on the well-demonstrated adequacy of the spherical harmonic model.

Compared with Jupiter's gravitational attraction, the total Lorentz force magnitude at the k th resonance is $0.008 |1/(1-k)| (V/10 \text{ volt}) (\mu\text{m/s})^2 (2g \text{ cm}^{-3}/\rho)$, where s is the particle's radius and ρ is its mass density; this assumes that field emission does not limit the electric potential of the particle, for otherwise s would appear to the power -1 (refs 10, 16). The component driving the $k = +2$ Lorentz resonance is about one-tenth the total Lorentz force. At the other resonances further from the planet, electromagnetic forces are somewhat less important because the higher-order components of the magnetic field decrease more rapidly than gravity does and because the speed of the particle across the field is generally lower.

As with any simple resonance, a region surrounding the precise resonance location shows amplified motions because the frequency of the force nearly matches the natural frequency of the system's response. Figure 2b depicts this by plotting the response of the system to typical forcing terms across the region of Jupiter's ring; this response is $\sim S_k [n_k^2 - k^2(n_k - \Omega)^2]^{-1}$ for each of the components. Note that the low-order resonances are quite broad (for example, the resonant term for the $k = +2$ case is large throughout the region $1.3-1.5R_j$), whereas higher-order resonances are substantially narrower. We have confirmed the results described above through a numerical integration of the full equations of motion (see ref. 14).

Planetary ring form and resonances

A recent reanalysis^{10,12} of the Voyager jovian ring images has identified three components to the system (see Fig. 2a). The main ring has an abrupt (<200 km wide) outer boundary whose half-power point is at $1.8086 (\pm 0.0014) R_{21}$, just outside the

small satellite Adrastea's orbit (semi-major axis $a = 1.8065 R_{J_1}$). Its low optical depth ($\tau \approx 10^{-5}$ – 10^{-6}) means that particle-particle collisions are rare^{10,12}. As seen in the back-scattered light that emphasizes large bodies, the main ring is <30 km thick¹⁰. A vertically extended halo, symmetrical about the ring plane, starts exterior to the ill-defined inner edge of the main ring at $\sim 1.71 (\pm 0.01) R_{J_1}$, only to disappear somewhere between 1.3 and $1.4 R_{J_1}$. The optical depth of the halo is comparable with that of the main ring and the particle size distribution there, as in the main ring, is a power-law starting at a few tenths of a micrometre. The brightness of a newly-discovered 'gossamer' ring^{12,27} decreases linearly with distance from the main ring and has a mean value $\sim 5\%$ that of the main ring. It stretches perhaps as far out as Thebe ($a = 3.15 R_{J_1}$) before vanishing from sight; its only notable feature is a $\geq 20\%$ enhancement at R_{syn} .

Because gravitational resonances, as distinct from Lorentz resonances, are so important in defining the Saturn ring structure^{3,4}, we consider their possible connection to the form of Jupiter's ring. Potentially significant gravitational resonances include Amalthea's 5:3 resonances¹¹, which are very near the main jovian ring's outer edge and the orbit of Adrastea: using the formalism of Shu²⁰ with values of J_2 and J_4 given earlier, we locate the horizontal (or eccentricity) resonance at $1.8054 R_{J_1}$ and the vertical (or inclination) resonance at $1.8126 R_{J_1}$; the mixed resonance is at $1.8071 R_{J_1}$. The third of these lies marginally within the positional error of the outer ring edge. The 5:3 resonance is very weak because of the small size of Amalthea coupled with the second-order nature of this resonance; its strength is only $\sim 10^{-5}$ of Mimas's 2:1 resonance which defines the outer edge of Saturn's B ring at the Cassini division, and 3×10^{-5} of Janus's 7:6 resonance which prevents the outer edge of Saturn's A ring from spreading²⁸. Even weaker is Io's 6:1 resonance, which is nearby¹¹. In addition, these resonances are unlikely to be important as one should not expect density waves to be generated, nor the shepherding mechanism to work, in the ethereal Jovian ring¹⁰. More probably, the boundary of the jovian ring is determined by the radial extent to which collisional ejecta is shed from Adrastea¹⁰, before the debris starts to evolve inwards. Nevertheless, the possible connection of this weak resonance with the form of the ring bears close scrutiny during the forthcoming Galileo observations for, if verified, it would require a re-evaluation of the mechanism whereby resonances terminate rings²⁹. For completeness we point out that Callisto's apsidal resonance is located at $1.39 R_{J_1}$, in the general vicinity of the inner perimeter of the halo. Note that the embedded satellites Adrastea and Metis have many resonances throughout the main ring.

Lorentz resonances, probably because they act on small grains, seem to account for much of the structure of the jovian ring. The jovian ring halo seems to be bounded by the interior $k = +3$ and $k = +2$ resonances. The outer boundary is easily understood. The orbits of grains in the main ring are expected to evolve rapidly inwards towards the halo because of plasma drag¹⁰. Once particles drift into the resonance zone they receive repeated out-of-plane thrusts and are pushed onto inclined (vertically extended) orbits. All halo particles reside in the region between the $k = +2$ and $k = +3$ resonances (see Fig. 2b); their jumbled trajectories have substantial radial and vertical excursions^{14,15}. The ring may appear to vanish at the strong $k = +2$ resonance because (1) the inward-evolving particles are so widely dispersed, having been scattered by both the $k = +2$ and $k = +3$ resonances, or (2) their extremely large excursions produce collisions into the jovian atmosphere, or (3) the individual particles have by this point simply evanesced through sputtering by magnetospheric particles¹⁰.

As already discussed, the main ring's outer boundary is probably associated with Adrastea, and not with Lorentz resonances. Nevertheless, despite the motion induced by the $k = +1$ and $k = +2$ terms, the overlapping inner $k = +3$ and $k = +4$ resonances produce a deep relative minimum in this locality (see Fig. 2b). The reduced vertical displacements here mean that the ring particles are more densely packed; this may possibly allow them

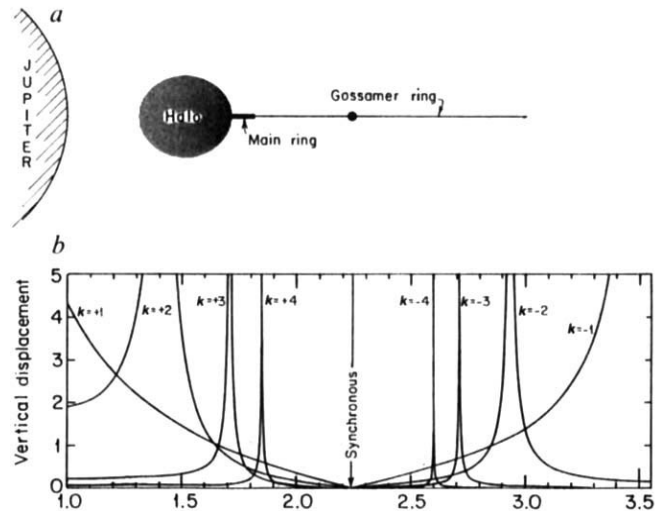


Fig. 2 Jovian ring boundaries are compared with positions of some low-order Lorentz resonances. *a*, A cross-section of Jupiter's ring, as deduced by Showalter^{10,12}. The thin main ring is $\sim 20\times$ as bright as the newly discovered gossamer ring²⁷, whose only feature is a slight enhancement at synchronous orbit (●). The locations of the outer edge of the gossamer ring and the inner edge of the halo are not well determined. The precise cross-section and density distribution of the halo are poorly constrained. *b*, Responses to the low-order Lorentz force components across the region of the ring in arbitrary units. The $k = +2$ and $+3$ resonances seem to bound the halo, and the $k = -2$ resonance may limit the outer edge of the gossamer ring.

to reach great enough number densities so as to act collectively in shielding themselves from the ambient plasma^{10,12}. Interparticle shielding in a dusty plasma can be such that the average charge on any particle is substantially less than that carried by an isolated grain (whose electric potential would be approximately the plasma's kinetic energy)^{16,17,30}. This reduced charge further lessens vertical motions and augments interparticle shielding¹². Perhaps this process could account for the surprising thinness of the main ring.

The brightness enhancement at R_{syn} in the gossamer ring may have three causes. First, vertical motions resulting from Lorentz forces will be limited in this region, even for the very tiny motes in this ring²⁷, as resonant forces are very weak near R_{syn} where $k \rightarrow \infty$ (see equation (6) and Fig. 2b). For shallow viewing angles such as those used by Voyager, this produces an enhancement at R_{syn} . Second, plasma drag, the principal cause of orbital evolution, vanishes at this point: hence any particles created by micrometeoroid collisions in this region will remain, whereas detritus elsewhere will be continually dragged away from its source region²⁷. Lastly, magnetically dominated particles have a tendency to drift towards synchronous orbit because of systematic charge variations^{16,17} (D. A. Mendix, personal communication).

The $k = -2$ resonance is near where the gossamer ring disappears. Perhaps outwardly-evolving particles are vertically dispersed enough by this resonance to make the already faint ring indistinguishable from background.

Lorentz resonances are much less important for Saturn's rings than for those of Jupiter because strengths are substantially lower (Table 1): Saturn's magnetic field is weaker as well as more regular than Jupiter's, and relative velocities are generally less because the ring straddles R_{syn} and the orbital velocity at R_{syn} is lower. In addition, saturnian ring particles are usually much larger (except for restricted regions^{3,4,10}) and charges smaller³⁰ so that charge-to-mass ratios are low. Furthermore, in the main ring system, optical depths are high so that our calculations, which ignore collisions, are moot.

The (interior) $k = +4$ resonance for Saturn is close to the B/C ring boundary ($1.525 R_{\text{A}}$) whereas the (exterior) $k = -3$ resonance lies just within the A ring edge ($2.267 R_{\text{A}}$) and the Keeler

gap ($2.263 R_n$). The (inner) $k = +4$ resonance is close to the gravito-magnetic stability limit for charged, massless grains launched with Keplerian velocity at $1.525 R_n$ (ref. 31) and the place where the magnetic equilibrium surface becomes undefined at $1.56 R_n$ (ref. 32); these apply, however, to particles for which q/m is effectively infinite.

It seems then that some part of the jovian ring's form is dictated by a resonance phenomena, albeit one different than the usual gravitational resonance of celestial mechanics. The

new resonance mechanism described here acts on small charged grains when they orbit through a non-uniform magnetic field. Additional study of the nature of this resonance will advance our understanding of the operation of all resonances.

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Strontium isotope evidence for preserved density stratification in the main zone of the Bushveld Complex, South Africa

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The Bushveld Complex formed from two isotopically distinct magmas. Magma addition and mixing can be tracked by measuring initial strontium isotope ratios of cumulates. Rocks with ratios of 0.7065 lie below the lower 2.2 km of the main zone (ratio = 0.7085), whereas those with ratios of 0.7073 lie above it. This unit represents a liquid layer intruded into the density-stratified magma pile near the base of the chamber. The Merensky Reef was produced by mixing of the pile with underlying trapped liquid enriched in precious metals.

EVIDENCE is accumulating that the layered rocks of the Bushveld Complex are the products of fractional crystallization from two magma series and their mixtures¹⁻⁵. Field and laboratory investigations have helped detect early derivatives of these series in the suites of quenched marginal rocks and related sills. The end-members have yet to be discovered. The stratigraphically older series were derived from SiO₂- and MgO-rich magmas similar to olivine boninites⁶; rocks of the other series are tholeiites. The lineages are termed U and A because of their ultramafic (orthopyroxene) and anorthositic (plagioclase) rock products, respectively⁷. U magmas are represented by quench-textured micro-orthopyroxenites, rich in incompatible elements (Zr = 70-300 p.p.m., Rb = 20-50 p.p.m.), 900-1,600 p.p.m. Cr and initial Sr ratio (R_0 ; ⁸⁷Sr/⁸⁶Sr₂₀₅₀) of 0.703-0.705 (refs 8, 9; Table 1). The marginal rocks quenched from magmas with a large A component are two-pyroxene microgabbros with low contents of incompatible elements (Zr ~ 15, Rb ~ 2 p.p.m.), ~250 p.p.m. Cr and R_0 values of 0.7065-0.7077. Rocks with compositions intermediate between these two 'end-members' are most abundant along the margin of the upper critical zone (Fig. 1) and are petrographically microgabbroic.

It is important to understand the mechanism of addition of parental magmas to the Bushveld chamber, because recent hypotheses concerned with the origin of layered complexes draw heavily on density stratification as a possible means by which

layering can be generated. Competing, yet (in many cases) overlapping hypotheses demand attention. Irvine and co-workers^{7,10} propose that rock layers grow laterally from the walls of magma chambers at shallow angles. The composition of each rock unit is controlled by that of its parental liquid layer, which forms part of a density-stratified system consisting of many such layers. Thus, new magmas may be intruded into stratified chambers at levels consistent with their density. Another school of thought¹¹ believes that such intrusion may occur as a turbulent plume which entrains existing magmas, spreads out in a discrete layer and controls sulphide precipitation. Most other hypotheses (see refs 12, 13 and A. J. Naldrett *et al.*, manuscript in preparation) depend on the new addition of magma forming a separate layer within a developing intrusion, at some level where a stratified magma system would have an appropriate density to accommodate the new liquid.

Unfortunately, definitive evidence in support of mechanisms such as density stratification, double-diffusive convection, turbulent plumes or other fluid-dynamic hypotheses in magma chambers has not been available. Evidence for the first of these possibilities is lacking because liquid layers are thought to be quickly destroyed during the processes of fractional crystallization and mixing, especially if they are thin as has mostly been assumed. This article presents field, geochemical and isotope evidence from the eastern Bushveld Complex for a 'fossilized'

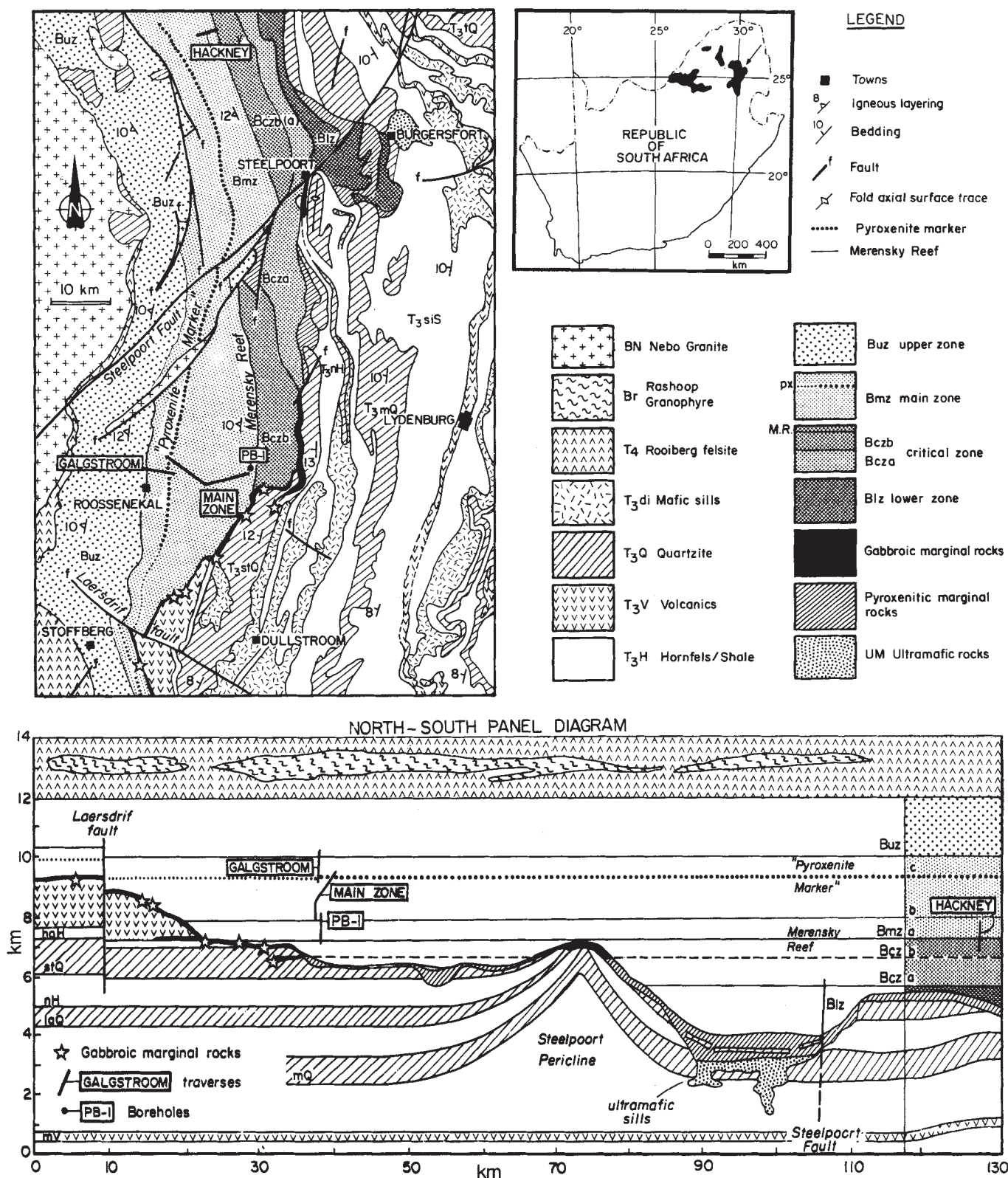


Fig. 1 Map of a portion of the eastern Bushveld Complex with a panel diagram from south to north along the contact region and stratigraphic column of the Bushveld Complex and Transvaal Sequence. Positions of sample traverses and gabbroic marginal rocks are annotated. Codes for lithologies follow ref. 1.

2–3-km thick layer of gabbroic magma within the 9-km thickness of layered rocks, which differentiated *in situ* with relatively minor mixing between the under- and overlying magma columns.

Geochemistry

Three hundred and fifty cumulate rock samples were collected from the eastern Transvaal and analysed by X-ray fluorescence

for major elements and 19 trace elements as part of a comprehensive study of the layered rocks of the eastern Bushveld Complex (see ref. 1 for analytical procedures). Fifty-five of these samples were analysed for Sr isotopes. Samples were selected to provide ample vertical coverage of the layered sequence, representative collections from each zone or group of petrographically related rocks and detailed coverage across important boundaries where

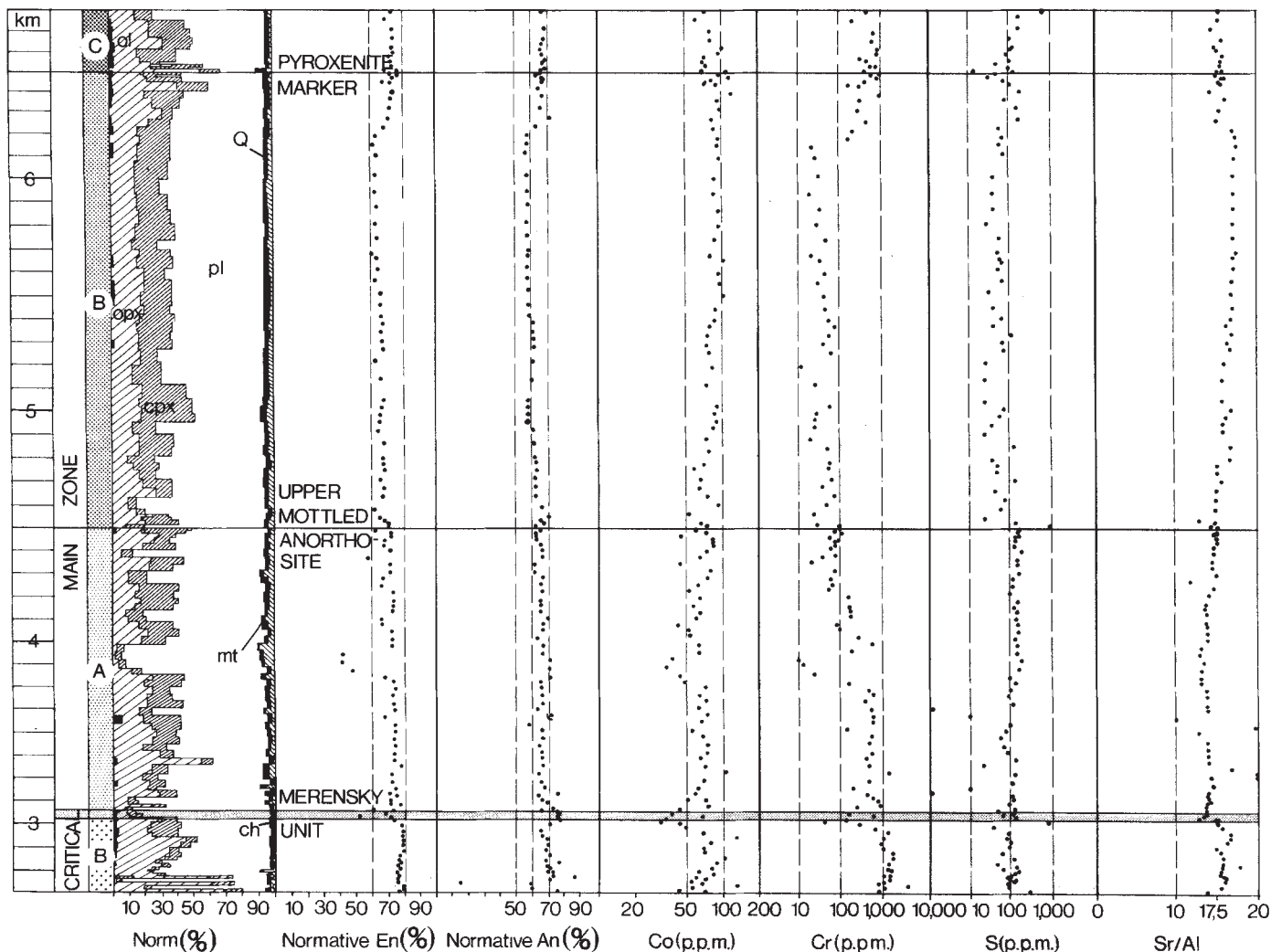


Fig. 2 Whole-rock geochemical data from 300 m below the Merensky Reef in subzone B of the critical zone to 300 m above the pyroxenite marker in subzone C of the main zone. Normative cumulus minerals: ch, chromite; mt, magnetite (above Merensky Reef); cpx, clinopyroxene; opx, orthopyroxene; pl, plagioclase; ol, olivine (Fe-rich); Q, quartz.

there are major changes in the cumulate parageneses. Thirty marginal rocks spanning the lower 5.5 km of the complex were also analysed (N. M. Evensen, B. V. Rao and M. R. S., in preparation). Concentrations of Rb and Sr were determined by isotope dilution.

The region of greatest interest lies between the Merensky Reef and the 'pyroxenite marker'^{14,15}, and comprises subzones A and B of the main zone (Fig. 2). In many respects, the main zone is an anachronism in the evolution of the complex. Its relatively homogeneous, two-pyroxene gabbros contrast in composition and lithology with the strongly layered units of the upper critical zone and the equally well-layered upper zone, which lie below and above, respectively. In the critical zone, the platinum group element (PGE) tenor of mineralized layers increases upwards, to climax in the UG2 chromitite layer and the Merensky Reef (Fig. 1). In the upper zone, abundant cumulus magnetite precipitated along with orthopyroxene, clinopyroxene and plagioclase, with Fe-rich olivine occurring at later stages. Whole-rock samples beneath the Main Magnetite Layer contain up to 5.6 p.p.m. total PGE¹⁶, with an average concentration of ~1.3 p.p.m.¹⁷.

Plots of norms, mineral compositions, some trace elements and Sr/Al ratio against stratigraphic height for subzones A and B of the main zone show major breaks at the base and top of this interval (Fig. 2). Discontinuities in the Pt/(Pt + Pd) ratio at the same levels were interpreted as evidence for addition of new magma¹⁷. At the pyroxenite marker the intruding liquid was 'replenished' in PGE, because the main zone is relatively dep-

leted compared with the critical and upper zones. The marker was originally considered as the expression of addition of fresh magma^{14,15}, even though supporting field relations like cross-cutting layers and footwall disturbances are ill-exposed or lacking in the eastern Bushveld. At the level of the Merensky Reef, however, there is much field^{1,9} and geochemical^{8,18} evidence for intrusion of new magma.

The lower 200 m of the main zone is lithologically variable. In the eastern Bushveld, the En content of orthopyroxene increases by up to 15 mol % in this interval (Fig. 2). Upward trends are fairly smooth, suggesting that simple fractional crystallization, with perhaps local addition of fresh magma (such as at the upper mottled anorthosite, Fig. 2) gave rise to gradually evolving profiles. Differentiation trends begin a marked reversal 300–500 m below the pyroxenite marker, but are most pronounced within metres of this layer. Other compositional tracers mirror these paths. Were subzones A and B of the main zone to be removed from the Bushveld Complex, it appears that the post-Merensky Reef and sub-pyroxenite marker compositions would prescribe a continuous trend, with only minor discontinuities between their whole-rock compositions. I suggest that this packet is an aberration on the normal fractionation sequence of critical zone to subzone C of the main zone and then upper zone. The petrographic and whole-rock composition suggests that the packet is a thick layer of liquid A that was intruded into the Bushveld chamber near its base, which has been preserved simply because of its thickness. However, mixing did occur at its lower and upper boundaries.

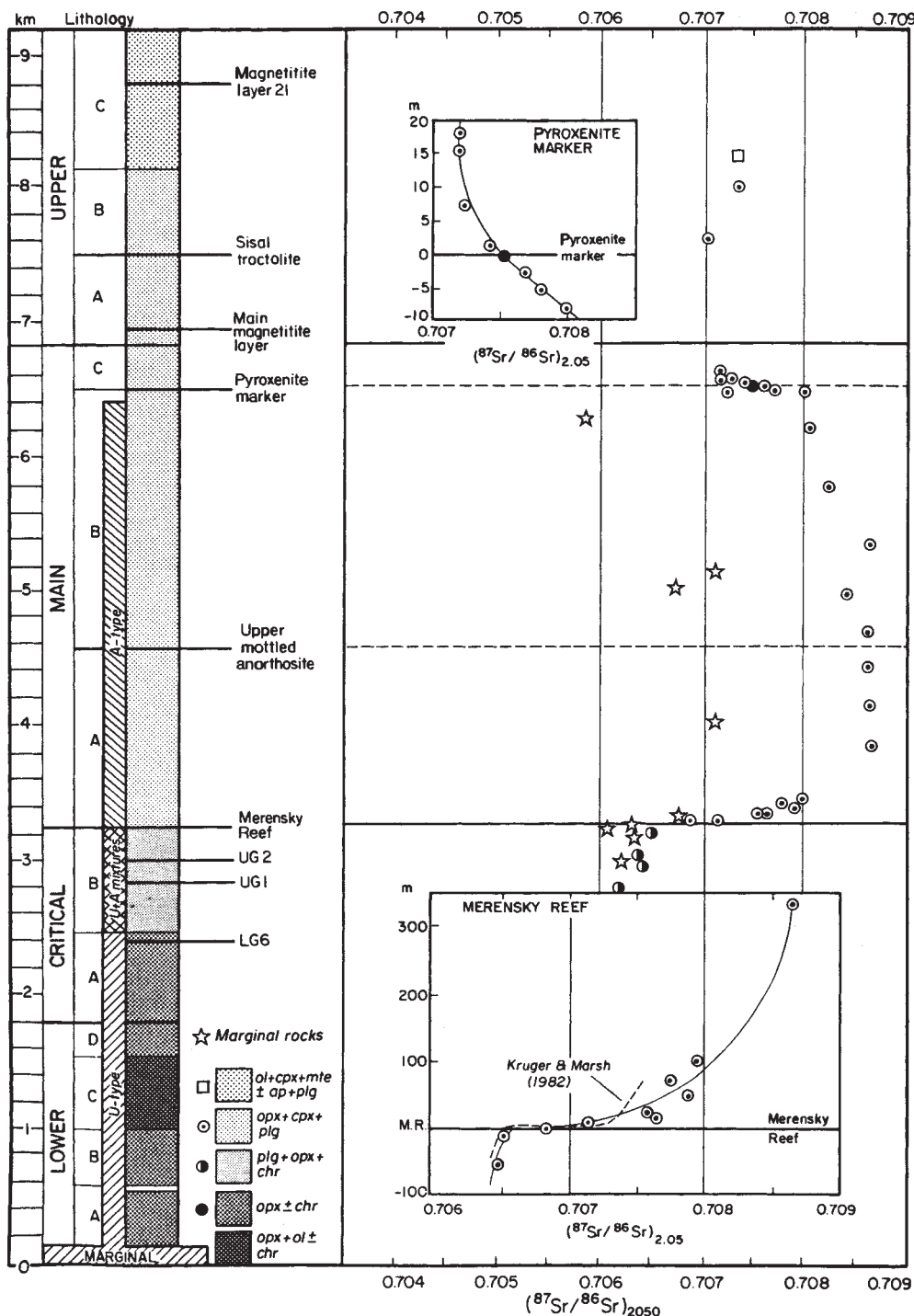


Fig. 3 $^{87}\text{Sr}/^{86}\text{Sr}$ at 2,050 Myr against stratigraphic height for the traverses and marginal rocks detailed in Fig. 1. Enlarged boxes show detail at the Merensky Reef and pyroxenite marker. Italicized mineral names are at levels of their addition (+) or deletion (-) from the cumulus assemblage.

Strontium isotope systematics

The Rb/Sr isotope age of the Bushveld layered rocks is 2,050 Myr. Early work gave an age of $1,954 \pm 30$ Myr on 13 samples²⁰, and Hamilton⁸ reported an average age of $2,095 \pm 24$ Myr. Both used the ^{87}Rb decay constant of $1.39 \times 10^{-11} \text{ yr}^{-1}$. Re-calculation of Hamilton's data with $\lambda^{87}\text{Rb} = 1.42 \times 10^{-11} \text{ yr}^{-1}$ gives an age of 2,050 Myr⁹. Unfortunately, because two magma types of differing R_0 are represented in the layered rocks, average ages such as these are less relevant than data from units of layered rocks which were derived from one or other of the magma types, or a stable mixture between them. Isochron ages of 2,048–2,057 Myr were acquired from sample sets from the lower zone, subzones A and B of the main zone, and the upper zone (M.R.S. *et al.*, in preparation). A neodymium age of $2,055 \pm 155$ Myr is reported by M.R.S. *et al.*, (in preparation). It seems that any Rb and Sr mobility must have operated homogeneously over cubic kilometre scales and is thus unlikely. The age of the

complex is also constrained by isotope age dating of rock units from the Transvaal Sequence into which the complex is intruded, and the Bushveld granite, which cuts the layered rocks. The most recent data have been compiled by von Gruenewaldt *et al.*¹⁹.

All Bushveld marginal rocks have R_0 values higher than expected from normal mantle (Rb/Sr = 0.03) at 2,050 Myr; the values range from 0.704 (U > A) to 0.7077 (A > U). Layered rocks have a wider range (0.704–0.7086). Such high values might originate in various ways, such as contamination on local or regional scales or Rb mantle enrichment. A brief treatment of Bushveld magma evolution is described here. Contamination of magmas has been proposed on the bases of trace element and isotope data^{1,3,8,9,20}. Because the R_0 ranges of marginal rocks and cumulates are approximately the same, local contamination of magmas in the chamber after quenching of the marginal rocks is considered unlikely. Regional contamination, however, is more probable, especially for the main zone, where values up

Table 1 Representative analyses of marginal rocks

	CO-211	CO-114	CO-071	CO-073
	Blz	Bcza	Bczb	Bmz
	U _{1a}	U >> A	A >> U	A ₁
SiO ₂	55.48	57.01	52.42	49.92
TiO ₂	0.28	0.40	0.79	0.35
Al ₂ O ₃	10.48	12.81	15.56	16.37
FeO	9.25	9.36	10.64	9.85
MnO	0.17	0.17	0.19	0.19
MgO	15.42	10.29	6.67	8.22
CaO	6.10	6.72	10.20	13.17
Na ₂ O	1.73	1.75	2.97	1.74
K ₂ O	0.76	1.23	0.32	0.11
P ₂ O ₅	0.05	0.09	0.15	0.01
Cr ₂ O ₃	0.22	0.11	0.04	0.04
NiO	0.06	0.05	0.04	0.03
(p.p.m.)				
Cr	1,349	765	297	246
Ni	403	218	111	135
Rb	30.7	47.3	4.17	1.69
Sr	159	199	247	323
Cu	60	63	29	13
Zr	236	105	137	12
Y	32	16	30	10
Eu*	0.2	-1.8	-5.4	1.7
Ce _N	30.3	48.2	44.6	4.4
Ce _N /Yb _N	7.4	9.1	3.9	1.2
En _{opx}	73.0	85.6	54.9	63.5
An _{plg}	61.2	ND	52.0	59.0
R ₀ at 2,050 Myr	0.70303 ± 6	0.70587 ± 9	0.70633 ± 4	0.70680 ± 4

Samples CO-211 and CO-073 are close to the end-member compositions; CO-114 is a fractionated derivative of U mixed with high- R_0 A liquid; CO-071 is A mixed with some fractionated liquid U. Analyses summed to 100% anhydrous; all Fe as FeO; ND, not determined; Eu*, chondrite-normalized Eu anomaly; R_0 , ($^{87}\text{Sr}/^{86}\text{Sr}$)_{2,050} (ref. 9 and M.R.S. *et al.*, in preparation); rare-earth element data from M.R.S. and M. P. Gorton (unpublished data). Blz, adjacent to lower zone; Bcza, adjacent to lower critical zone; Bczb, adjacent to upper critical zone; Bmz, adjacent to main zone.

to 0.7086 exceed those of possible local contaminants like the Rooiberg felsites ($R_0 < 0.708$; R. E. Harmer, personal communication). However, Pretoria Group shales²¹ have initial ratios between 0.708 and 0.723 (ref. 9) and so may be more likely candidates. Combined Nd and Sr isotope data (M.R.S. *et al.*, in preparation) indicate that both U and A magmas lie in the enriched quadrant of a $\epsilon_{\text{Nd}}/\epsilon_{\text{Sr}}$ plot. The U dominant rocks lie closer to the "Bulk Earth" composition, but increasing amounts of A drives points diagonally from the fulcrum towards $\epsilon_{\text{Sr}} = 150$, $\epsilon_{\text{Nd}} = -20$, typical of crustal material²². Thus, relatively homogeneous contamination on scales of hundreds of thousands of cubic kilometres is indicated.

The addition of liquid A to the Bushveld magma chamber has been monitored by making use of the widely contrasting R_0 values of the U and A magma series (ref. 9 and M.R.S. *et al.*, in preparation). The Sr isotope data provide confirmation of the unusual character of the main zone (Fig. 3). R_0 values of cumulates increase from 0.7065 immediately below the Merensky Reef to 0.7085 about 200 m into subzone A of the main zone. The increase in R_0 across the Merensky Unit is sudden. It was first noted by Kruger and Marsh¹⁸ and equated with an addition of new magma with high R_0 . Geochemical parameters through the first 2.2 km of the main zone (Fig. 2) prescribe a trend suggestive of steadily increasing fractionation, on which broad cyclic sweeps are superimposed, particularly evident in the Co data. Samples from subzone A of the main zone have constant R_0 values, regardless of the irregularities in the trends. In subzone B, which has somewhat more regular data for Co, the isotope data show local decreases. I do not dismiss the possibility, however, that high R_0 magma was added intermittently within the main zone. In the limitations of sample

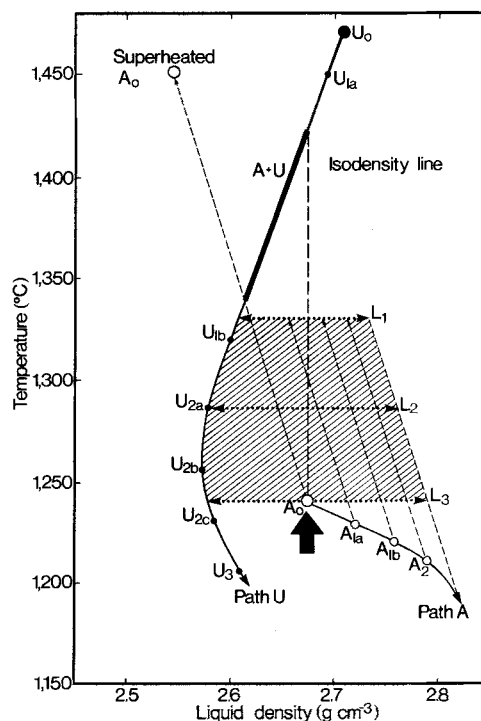


Fig. 4 Variation in liquid density in relation to temperature for Bushveld parental magmas at 5 kbar. The range of densities expected in the chamber are controlled by initial densities (points U_0 and A_0), upon which are superimposed the effects of fractional crystallization of different minerals. The crystallization of orthopyroxene from U liquids along path (U_0-U_3 , where subscript refers to derivative type) will decrease liquid density until plagioclase begins to crystallize (near U_2), whereupon density increases, assisted by late-stage iron enrichment. A liquids increase in density with temperature drop during plagioclase crystallization (path A). At 1,330 °C (CO-114 liquidus temperature at 1 atm² + 10 °C kbar⁻¹) the chamber might be expected to contain liquids with a wide range of densities, comprising U derivatives, superheated A derivatives and A + U mixtures, located along line L_1 . Further temperature drop will increase that range through L_2 to L_3 . New liquids could readily be accommodated at levels controlled by their densities, like at point A_0 , in the middle of the density range (solid arrow). Thick line A + U represents the temperature/density range over which U derivatives and superheated A derivatives might finger mix; thin dashed lines are superheating paths for A derivatives.

coverage, values of R_0 remain approximately steady through the bulk of the main zone, although slight fluctuations do begin 300–500 m below the pyroxenite marker, where other indices also become perturbed. At the same distance below the marker, R_0 decreases to 0.708, but the most marked decrease, to 0.7073, occurs within a 25-m interval centred on the marker. The R_0 remains at ~0.7073 through the rest of the complex.

These data show that the main zone represents a slab of layered cumulates that were derived from high R_0 liquid. Its basal portions mixed with the underlying post-Merensky Reef U + A residuum to produce a steep R_0 gradient. Similarly, the less rapid R_0 drop at the top of subzone B can be equated with deeper penetration of the front along which A and A + U magmas mixed. Possibly the pyroxenite marker might be considered as a sort of semipermeable membrane along the interface between 'old' and 'new' magmas. The post-Merensky Reef and pyroxenite marker R_0 values are almost contiguous: the difference of 0.0005 between the 0.7065 value at the Merensky Reef and 0.707 at the pyroxenite marker may be ascribed to upward transfer of some A residuum from the crystallizing subzones A and B of the main zone.

Mass-balance calculations can be made to determine U:A ratios at the time of formation of the Merensky Reef and the upper zone, assuming no local contamination further affected

Table 2 Rb, Sr and isotope data in relation to stratigraphic height

Sample cumulates	Height above M. Reef (m)	Rb	Sr	$^{87}\text{Rb}/^{86}\text{Sr}$	$^{87}\text{Sr}/^{86}\text{Sr}$	$(^{87}\text{Sr}/^{86}\text{Sr})_{2050}$
BDS-120	5,040	1.11	281.1	0.0114	0.70766 $\pm$ 4	0.70732
BDS-87	4,740	4.00	292.5	0.0396	0.70856 $\pm$ 8	0.70739
BDS-82	4,450	1.94	205.4	0.0273	0.70785 $\pm$ 5	0.70704
BDS-124	4,140	19.02	447.8	0.1229	0.71104 $\pm$ 2	0.70741
BDS-54	3,860	1.80	400.0	0.0130	0.70786 $\pm$ 6	0.70748
BDS-62	3,258	4.31	214.0	0.0583	0.70891 $\pm$ 3	0.70719
BDS-69	3,255	1.54	241.7	0.0184	0.70774 $\pm$ 5	0.70720
BDS-68	3,248	2.11	247.3	0.0247	0.70794 $\pm$ 3	0.70721
BDS-67	3,241	2.94	331.7	0.0256	0.70819 $\pm$ 5	0.70743
BDS-66	3,240	4.82	96.5	0.1446	0.71180 $\pm$ 2	0.70753
BDS-65	3,238	2.51	314.6	0.0231	0.70839 $\pm$ 3	0.70771
BDS-64	3,235	1.83	267.5	0.0198	0.70837 $\pm$ 5	0.70779
BDS-63	3,232	1.84	309.1	0.0172	0.70851 $\pm$ 5	0.70800
BDS-30	3,140	2.42	256.5	0.0273	0.70808 $\pm$ 3	0.70727
BDS-35	2,940	1.87	280.9	0.0193	0.70881 $\pm$ 3	0.70824
BDS-41	2,530	3.06	289.2	0.0306	0.70911 $\pm$ 2	0.70821
BDS-09	2,140	3.18	296.3	0.0311	0.70953 $\pm$ 6	0.70861
BDS-17	1,690	7.15	321.1	0.0644	0.70994 $\pm$ 6	0.70804
BDS-49	1,410	8.42	275.5	0.0885	0.71123 $\pm$ 4	0.70862
PB1-383	1,220	6.97	270.9	0.0745	0.71077 $\pm$ 2	0.70857
PB1-526	840	5.79	219.9	0.0762	0.71090 $\pm$ 2	0.70865
PB1-953	330	5.77	235.5	0.0709	0.71075 $\pm$ 3	0.70866
BDS-216	100	1.76	284.9	0.0179	0.70845 $\pm$ 3	0.70792
PB1-1256	70	9.57	342.3	0.0809	0.71013 $\pm$ 3	0.70774
BDS-219	50	1.81	364.3	0.0144	0.70832 $\pm$ 6	0.70790
PB1-1282	25	2.31	349.9	0.0191	0.70816 $\pm$ 3	0.70760
BDS-218	18	5.24	279.1	0.0543	0.70926 $\pm$ 6	0.70766
PB1-1319	4	3.97	380.2	0.0302	0.70802 $\pm$ 3	0.70713
PB1-1332	0	2.08	375.0	0.0160	0.70731 $\pm$ 3	0.70684
BDS-220	-15	0.73	305.4	0.0069	0.70761 $\pm$ 5	0.70651
BDS-202	-55	0.53	293.8	0.0052	0.70665 $\pm$ 2	0.70650
MDH5-202	-320	1.39	373.3	0.0108	0.70672 $\pm$ 3	0.70640
MDH24-79.1	-500	0.55	324.6	0.0049	0.70634 $\pm$ 2	0.70620
Marginal rocks						
CO-059	3,090	15.65	353.2	0.1282	0.70964 $\pm$ 7	0.70585
CO-052	1,800	1.76	405.2	0.0126	0.70754 $\pm$ 5	0.70717
CO-048	1,740	4.06	338.2	0.0347	0.70773 $\pm$ 2	0.70670
CO-073	~0	1.69	340.4	0.0144	0.70722 $\pm$ 4	0.70680
CO-250	~0	3.22	341.5	0.0273	0.70710 $\pm$ 2	0.70629
CO-251	~0	4.41	325.4	0.0392	0.70720 $\pm$ 1	0.70604
CO-071	-40	4.17	273.8	0.0441	0.70763 $\pm$ 4	0.70633
CO-089	-360	2.13	352.0	0.0175	0.70667 $\pm$ 2	0.70615

$^{87}\text{Sr}/^{86}\text{Sr}$ ratios are given at the 1σ level. Analyses of NBS-987 are reported as 0.71026 ± 3 (1σ). Boxed samples: Merensky Reef (PB1-1332) and pyroxenite marker (BDS-66).

the regionally contaminated magmas. The crystallization rate and bulk D of the rocks below the Merensky Reef cannot be estimated at present without excessive speculation. It is assumed that U liquids had $R_0 \sim 0.704$ and Sr = 167 p.p.m. (sample CO-211; ref. 9), quenched liquids at the top of the critical zone have $R_0 = 0.7065$ and Sr = 330 p.p.m. (CO-250; ref. 9) and quenched liquids from which the upper zone crystallized had $R_0 = 0.707$ with Sr = 380 p.p.m. (CO-052). The pure A liquids (main zone) had $R_0 \sim 0.7085$ and high Sr (> 450 p.p.m.). Using the equations of De Paolo²³, adapted for simple mixing, it can be calculated that the Merensky Reef crystallized from a total mixture of 88% U + 12% A. A further 25% of fractionated A residuum added to the elevated Merensky Reef source magma simulates the R_0 of the upper zone, which crystallized from a total U:A mixture of 71:29.

Isotope relations

Cumulates of subzones A and B in the main zone have $R_0 \sim 0.7085$ (Fig. 3). The marginal rocks that occur along the basal contact of the main zone (Fig. 1) have $R_0 = 0.706-0.7075$ (ref. 9). The isotope ratios of marginal and main zone layered rocks are completely out of equilibrium. The marginal rock ratios closely resemble those of layered rocks from the upper critical zone (0.7061-0.7065; Table 2) and the upper zone (0.7073). They

are probably the quenched equivalents of the magma(s) that occupied the chamber at Merensky Reef times. Derivatives of these magmas, modified at a later stage by addition of main zone residual liquids, crystallized rocks that now occur ~3 km higher in the succession.

The marginal rocks along the basal contact of the main zone occur as a thin tenuous swath, some 10% of the extent of the upper critical zone marginal zone. Field relations⁹ suggest that the intrusion of A liquids that were to give rise to the main zone practically destroyed the original marginal rocks and replated the basal margin with some high- R_0 material. Two marginal rock generations occur south of the Laersdrif Fault (Fig. 1), where magnetite-rich microgabbros are intruded by fine-grained, two-pyroxene gabbro sheets (J. Schweitzer, personal communication).

Density considerations

The lower subzones of the main zone crystallized as a sheet from A liquids that were added in copious amounts to the Bushveld magma chamber, which was, at the time, crystallizing the upper critical zone from U >> A liquids. The massive addition of A liquid occurred near the base of the chamber, at a level consistent with its density. The magmas in the chamber were relatively hot yet dense; A magmas were cooler and slightly less

dense. The A sheet underwent limited mixing at its base with fractionated U + A mixture, giving rise to the steep R_0 gradient. The fact that the Merensky Reef is located at this level is surely more than coincidence, even though precious metals were steadily concentrated in residual magmas during formation of the lower and critical zones²⁴, as also shown by upward increases in the PGE tenor of the chromitite layers. I argue that evolution of the Merensky Reef is inextricably bound to the addition of A magma.

I performed melting experiments on rocks considered to be quenched $U \gg A$ (CO-114) and $A \gg U$ (CO-071) mixtures⁵ (Table 1). Their liquidus temperatures were 1,275 and 1,210 °C, respectively. The amount of chromite precipitated on mixing two such magmas depends on their proportions and differences in fO_2 (ref. 5). Because the critical zone contains abundant chromitite layers up to 1.6 m thick and mineral compositional variations across such layers are relatively minor (C. J. Hatton, unpublished data), it can be assumed that the volumes of magma A intermittently added to the magma chamber were small (supported by the isotope data), but their oxygen fugacities were very different. The pre-Merensky Reef rocks retain evidence of continuous sulphur saturation of the parental magmas²⁴, yet sulphides in the main zone are extremely rare. The bulk compositions of A and U magmas were dissimilar. Addition of 2–3 km of magma A would have increased pressures at the top of the critical zone by ~1 kbar. I suggest that the changes in T , P , X , fO_2 and fS_2 imposed on the trapped packet of U + A magma mixtures from which the upper critical zone was crystallizing were sufficient to stimulate the rapid precipitation of olivine and sulphides rich in precious metals, so giving rise to the Merensky Reef.

At the top of the A sheet, the cool dense A was overlain by hotter, less dense 88% U + 12% A, stable conditions ideally suitable for diffusive mixing. Less dense fractionated residua from crystallization of the A sheet were added to the overlying magma column, causing the downward migration of the interface controlled simply by volume changes and mass diffusion. The liquids lying above the pyroxenite marker were homogenized to $R_0 = 0.7073$. Perhaps the abundant upper zone magnetite (~10 modal %) is a signature of the upward transfer of Fe-enriched residual material.

Density relations of some members of the U and A families have been investigated²⁵. Figure 4 represents conditions in the magma chamber at 5 kbar pressure²⁶ before intrusion of A liquids. Liquids with different densities would be present, density contrasts having a greater range with falling temperature. New A_0 liquid or its derivatives could intrude readily the stratified pile at a level controlled by its density, and elevate the superincumbent liquid column, still enriched in precious metals, by 2–3 km.

Conclusions

Subzones A and B of the eastern Bushveld Complex represent a frozen interval in its evolution, where a massive inflow of gabbroic liquid into the magma chamber, at a level consistent with its density, split the crystallizing column of U + A liquids. Figure 5 describes the strontium isotope evolution of the eastern Bushveld Complex by a scheme of magma addition and mixing. In simple terms, the elevation of the post-Merensky Reef residuum gave rise, with later mixing at the top of the A sheet, to the pyroxenite marker, subzone C of the main zone and the upper zone. Mixing at the base of the sheet was over a 200–300-m interval, which created a pronounced R_0 gradient, along with lithological and compositional fluctuations. Marginal rocks with isotope ratios out of equilibrium with adjoining cumulates attest to this intrusion mechanism. Minor addition of A liquids occurred in the crystallizing sheet. Thus, the magma generation and intrusion chronology of the Bushveld Complex is simplified and Occam's razor rediscovered.

This is the first evidence of fossilized density stratification in a large layered intrusion. It provides strong evidence that double-diffusive convection and magma mixing were important processes in the evolution of the Bushveld Complex.

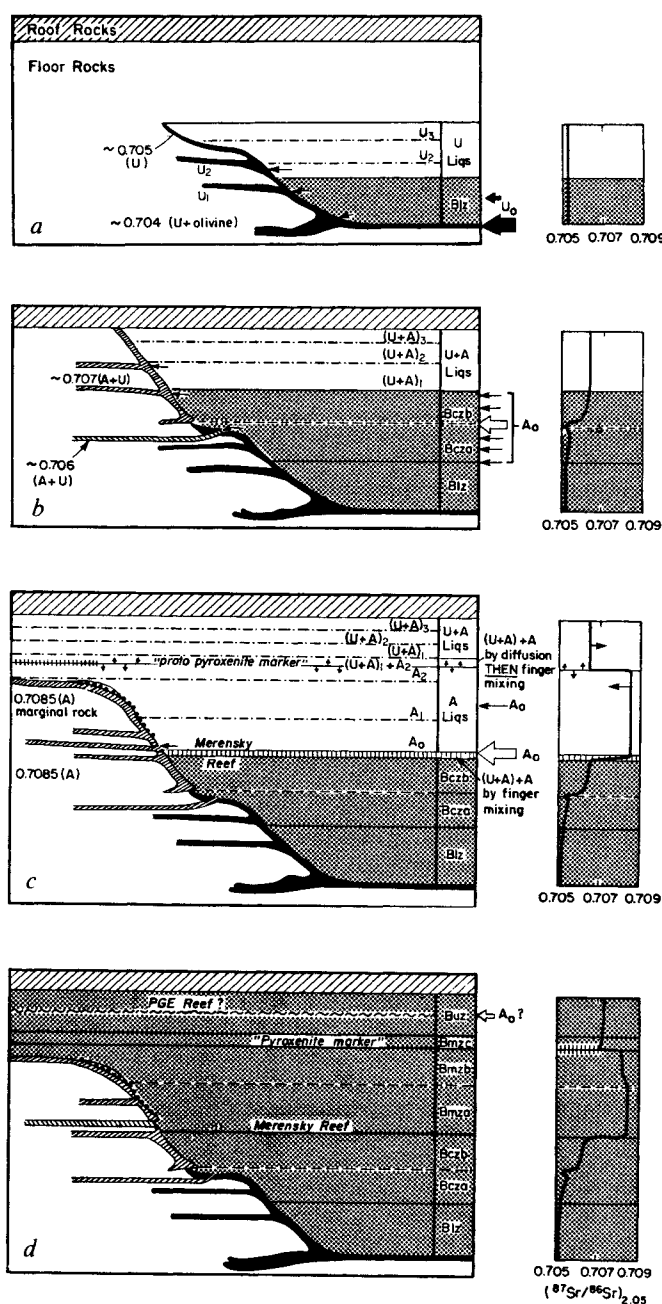


Fig. 5 Diagram of the possible sequence of events that gave rise to the Sr isotope profile of the eastern Bushveld complex (M.R.S. *et al.*, in preparation). *a*, U_0 magmas are intruded into the chamber and differentiate, giving cumulates, U derivatives and related sills with primitive R_0 (0.703–0.704). *b*, Addition of A_0 liquids commences during lower critical zone times, with major addition at subzone A–B boundary. R_0 in cumulates and residual $U \gg A$ mixtures increases steadily to 0.7065. Sills of U + A magmas are intruded into the floor rocks. *c*, Massive addition of A_0 occurs near the base of the chamber and splits the U + A liquid pile. Mixing with basal trapped liquid makes the Merensky Reef. The proto-pyroxenite marker forms. The R_0 of precipitating cumulates increases dramatically to 0.7085. *d*, Crystallization of the A sheet continues. Transfer across the proto-pyroxenite marker decreases in cumulates of subzone B to 0.708 and increases R_0 in subzone C of the main zone and the upper zone to 0.7073.

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Identification of serotonin M-receptor subtypes and their specific blockade by a new class of drugs

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We describe a new class of drugs that selectively block serotonin M-receptors on peripheral neurones. Because of their high affinity, some of these drugs are the most potent of any pharmacological class yet reported. They have allowed the identification of three M-receptor subtypes, one of which is responsible for mediating the painful effects of serotonin in humans.

GADDUM and co-workers suggested the existence of different serotonin (5-hydroxytryptamine, 5-HT) receptor subtypes when they showed that this substance contracts the guinea pig ileum by two different mechanisms: a direct action on the smooth muscle and an indirect action mediated by the release of acetylcholine from parasympathetic nerve endings. Because the former mechanism was blocked by dibenzylamine (D) and the latter by morphine (M), the excitatory 5-HT receptors on smooth muscle and parasympathetic nerve endings were designated D- and M-receptors respectively^{1,2}. Neither dibenzylamine nor morphine is selective or potent in its antagonism of 5-HT. In fact morphine prevents the indirect contractile action of 5-HT in this preparation not by a specific blockade of neuronally located receptors, but by a non-selective inhibition of acetylcholine release^{3,4}.

Although many potent new 5-HT antagonists for smooth muscle D-receptors have subsequently become available, they are all completely inactive at M-receptors located on peripheral neurones. Some structural analogues of (–)cocaine, which inhibits the neuronal actions of 5-HT on the guinea pig ileum^{1,2,5} and sympathetic ganglia^{6,7}, and metoclopramide behave as competitive antagonists at these sites^{8–11}. These compounds, however, have several other pharmacological properties and display only limited potency and selectivity for M-receptors^{10,12,13}. Thus, despite the wide distribution of excitatory 5-HT receptors in the peripheral nervous system¹⁴, their pharmacological and functional characterization has been greatly hindered by the lack of potent, selective agonists and antagonists. We have adopted an approach analogous to that originally used for generating selective antagonists for β -adrenoreceptors and histamine H₂-receptors: the naturally occurring ligand, in this case 5-HT, was used as the starting point for chemical derivation^{15–17}. Highly selective agonists for either D- or M-receptors were synthesized and extremely potent and selective antagonists of

neuronal 5-HT M-receptors were produced. Large differences in the affinity constants obtained for these antagonists on different isolated tissues containing neuronal excitatory 5-HT receptors show that there are at least three pharmacologically distinguishable M-receptor subtypes. Preliminary reports of some of these findings have been reported elsewhere¹⁸.

Assay systems

Quantitative responses to the newly synthesized 5-HT analogues were routinely obtained in six different *in vitro* assay systems. A minimum of three independent experiments were conducted for each compound and complete concentration–response curves were constructed. To assess their affinity for M-receptors three bioassays were used: the de-sheathed rabbit vagus nerve, the isolated rabbit heart, and the guinea pig ileum incubated in the presence of 10^{–6} M methysergide (see Figs 1, 2 legends). In the first two assay systems 5-HT D-receptor antagonists, such as methysergide (10^{–5} M, *n* = 3, data not shown), have no influence on the action of 5-HT and in the third assay, using guinea pig ileum, 10^{–6} M methysergide is continuously present to abolish the direct action of 5-HT on the smooth muscle D-receptors present in this preparation. We also used three non-M-receptor systems. Contractile responses of rat uterine strips were used to assess the affinity of compounds to smooth muscle 5-HT D-receptors. In addition, displacement of the specific binding of [³H]5-HT or 2-[¹²⁵I]iodo] lysergic acid diethylamide ([¹²⁵I]LSD) from rat cortex membranes was used for measuring the affinity of compounds to central 5-HT₁ and 5-HT₂ receptors, respectively^{19,20}.

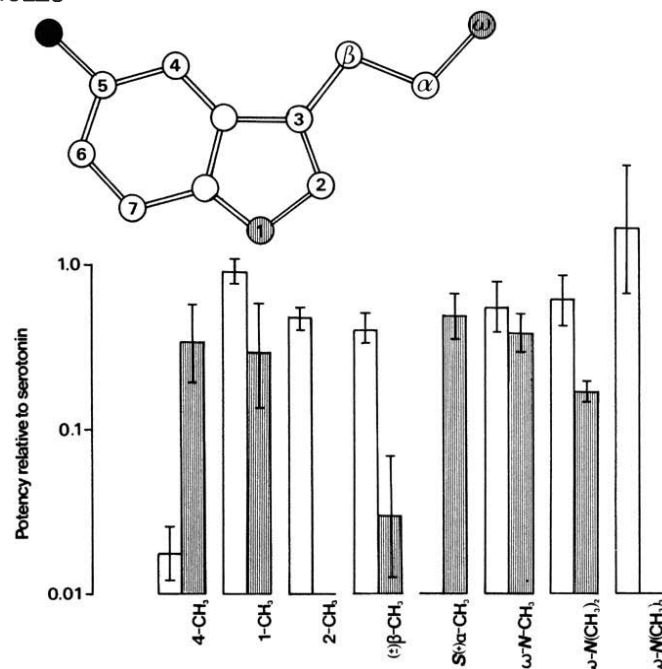
Selective agonists for M- and D-receptors

Our initial chemical programme was based on the assumption that different conformations of the 5-HT molecule are required for activation of M- and D-receptors. By systematic methyl substitutions in the indole nucleus and ethylamine side chain we attempted to reduce the conformational freedom of the 5-HT

* Deceased.

Fig. 1 Agonist potency of methylated 5-HT analogues relative to 5-HT (potency = 1.0) on the de-sheathed rabbit vagus nerve (open columns) and the rat uterus (striped columns). Each column indicates the geometric mean value obtained from at least three independent experiments. Vertical lines on each column indicate 90% confidence limits.

Methods. Isolated rabbit vagus nerve. New Zealand White or Yellow Silver male or female rabbits weighing 3.0–4.0 kg were killed by the intravenous injection of pentobarbital. Both vagi were dissected out, de-sheathed (that is, epineurium removed) and placed in a small compartmentalized Perspex chamber containing physiological salt solution as described previously^{37,38}. Extracellular recordings of the compound action potential were made so that the amplitude of the action potential in both myelinated (α and $\alpha\beta$) and non-myelinated (C) fibres could be recorded separately³⁸. 5-HT caused a dose-dependent reduction in the amplitude of the 'C' fibre action potential at concentrations between 3.5×10^{-7} and 2.5×10^{-6} M without affecting the amplitude of the action potential carried in the faster-conducting α and $\alpha\beta$ fibres. The maximal reduction in the 'C' fibre action potential amplitude caused by 5-HT was $45 \pm 0.4\%$ ($n = 35$), indicating that a subpopulation of 'C' fibres is preferentially affected by 5-HT. Based on these results, 45% reduction in amplitude was considered the 'maximal' (100%) effect. The relative potency of the methylated analogues was measured by running a full cumulative concentration-response curve for 5-HT first and then washing out 5-HT for as long as required for the amplitude of the 'C' fibre action potential to return to its original value (usually 30–40 min). The contact time for each concentration of agonist was 15 min. Methylated analogues were tested subsequently in the same way. Their potencies relative to 5-HT at 50% of the maximum 5-HT effect were then assessed from these dose-response curves for each experiment. Each analogue was tested for antagonistic action of the 5-HT effect by combining the highest concentration which produced no agonist action on its own with 1.5×10^{-6} M 5-HT. None of the analogues had antagonist action when tested in this way. To demonstrate that the reduction in amplitude of the 'C' fibre action potential caused by each of these analogues really resulted from a stimulation of 5-HT M-receptors and not from some other mechanism, the ability of a selective 5-HT M-receptor antagonist to block a maximally effective concentration of each analogue was tested, as such antagonists became available. The agonist action of all analogues was blocked by prior incubation of the nerve with ICS 205-930 (10^{-8} M) for 30 min ($n = 4$). Isolated rat uterus. To study the action of these analogues on D-receptors, isolated uterine strips from oestrogen-treated rats were used as previously described³⁹. Full concentration-response curves for the contractile action of 5-HT were constructed in each case (3×10^{-9} – 3×10^{-6} M). Uteri that did not respond to 10^{-8} M 5-HT were discarded. When the concentration of 5-HT producing a maximal contraction had been established, it was washed out and the methylated analogue tested in the same way. The relative potencies of the methylated analogues were again estimated from the dose-response curves at 50% of the maximal 5-HT effect. As in the vagus nerve, each analogue was tested for antagonism of the 5-HT effect by combining the highest concentration which produced no agonist action on its own with 10^{-6} M 5-HT. None of the analogues had antagonist action when tested in this way. The agonist action of each of the methylated analogues was abolished by prior incubation of the muscle strips with methysergide (10^{-6} M) for 30 min ($n = 6$).



molecule and thereby create analogues with increased specificity for one of these receptors.

Figure 1 shows that this strategy indeed provided highly selective D- and M-agonists. For instance, 2-methyl-5-HT is 2,000 times less potent than 5-HT as an agonist at D-receptors in the rat uterus and yet still possesses ~50% of the potency relative to 5-HT at M-receptors in the vagus nerve. Conversely, *S*(+) α -methyl-5-HT has ~50% of the potency of 5-HT in the uterus, but is ~770 times less potent than 5-HT in the vagus nerve.

These two compounds were tested in the other four assay systems (Table 1). The 2-methyl analogue possesses ~50% of the potency of 5-HT on two other M-receptor systems, the rabbit heart and guinea pig ileum. There was no statistically significant difference between the relative potencies of this compound on the three M-receptor systems, indicating that these receptors may be identical. However, the data obtained with *S*(+) α -

methyl-5-HT suggests otherwise. Although this compound showed greatly reduced potency relative to 5-HT on all three M-receptor assays, there were differences between the values obtained in each system. Thus, *S*(+) α -methyl-5-HT was 770 times less potent than 5-HT on the vagus, 22 times less potent on the heart and only ~11 times less potent on the ileum. Subsequent work with competitive antagonists, as indicated below, confirmed that there are indeed different subtypes of M-receptors in these three tissues.

Radioligand binding data show that the affinity of *S*(+) α -methyl-5-HT and 2-methyl-5-HT to 5-HT₁ receptors was ~34 and 370 times, respectively, less than that of 5-HT itself, indicating that these receptors are different from both the D- and M-types (Table 1 and ref. 19). *S*(+) α -methyl-5-HT had an affinity similar to that of 5-HT in both the uterus and the competition binding studies using [¹²⁵I]LSD in rat cerebral cortex membranes, whereas 2-methyl-5-HT was devoid of

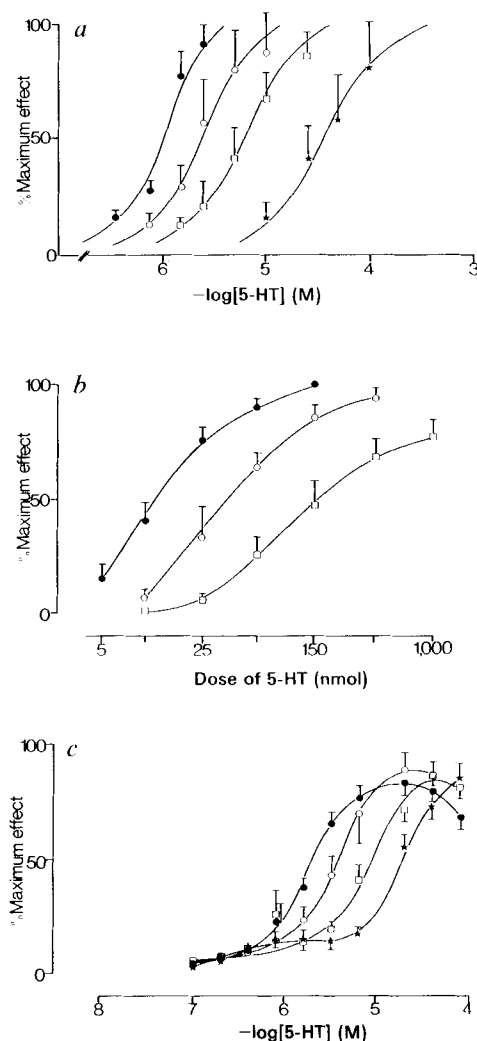
Table 1 Agonist potency of methylated 5-HT analogues relative to 5-HT (=100)

	Vagus nerve	M-receptors Heart	Ileum	D-receptors Uterus	Cortex-binding sites 5-HT ₁	5-HT ₂
5-HT	100	100	100	100	100	100
<i>S</i> (+) α -methyl-5-HT	0.13 (0.10–0.16)	4.6 (2.8–7.4)	8.9 (5.3–14.9)	49.1 (35.5–67.9)	2.9 (2.5–3.2)	83.5 (56.9–122)
2-Methyl-5-HT	47.3 (41.1–54.5)	52.6 (42.4–65.3)	44.8 (31.8–63.1)	0.05 (0.01–0.07)	0.27 (0.24–0.29)	3.5 (1.5–7.9)

Values are geometric means with 90% confidence limits obtained from a minimum of three independent assays. Methods for estimation of the relative potencies of analogues on the rabbit vagus nerve and rat uterus are given in Fig. 1 legend. Methods for measuring affinities to neuronal 5-HT receptors in the rabbit heart, guinea pig ileum and rat cortex membranes have been published previously^{19,20,34}.

Fig. 2 5-HT concentration-response curves in the presence and absence of competitive M-receptor antagonists. *a*, Rabbit vagus nerve; *b*, rabbit heart; *c*, guinea pig ileum. *a*, Ability of 5-HT to cause a reduction in the amplitude of the 'C' fibre action potential in the absence of antagonist (●) and after prior equilibration of the nerve with compound I (see Table 2) at concentrations of 2×10^{-13} M (○), 10^{-12} M (□) and 5×10^{-12} M (*); $n = 4$ in each case. In a separate series of experiments, it was shown that, unlike compound I, the 5-HT D-receptor antagonist methysergide (10^{-5} M) did not influence the 5-HT concentration-response curve in this tissue ($n = 3$, data not shown). *b*, Positive chronotropic effect of 5-HT in the absence of antagonist (●; $n = 9$) and after prior equilibration of the heart with compound II (see Table 2) at concentrations of 3×10^{-10} M (○; $n = 5$) and 10^{-9} M (□; $n = 7$). Unlike compound II, methysergide (10^{-5} M) had no effect on the 5-HT concentration-response curve in this tissue ($n = 3$, data not shown). *c*, Contractile action of 5-HT in the absence (●) and presence of compound III (see Table 2) at concentrations of 10^{-8} M (○), 3×10^{-8} M (□) and 10^{-7} M (*); $n = 4$ in each case. Vertical bars indicate the s.e.m. in each case.

Methods. *a*, In each tissue a cumulative concentration-response curve to 5-HT was established initially, as described in Fig. 1 legend. After washing out the agonist, the nerve was equilibrated with a set concentration of compound I for 60 min and the concentration-response curve to 5-HT was then repeated in the continued presence of the same concentration of compound I. When a concentration of 5-HT sufficient to produce a maximum effect had been applied, the antagonist combination was washed out, the nerve equilibrated for 60 min with a higher concentration of antagonist and the procedure repeated. *b*, Isolated rabbit hearts were perfused by the Langendorff technique and concentration-response curves were constructed for 5-HT in the absence and presence of compound II as described previously⁹. The antagonist was allowed to equilibrate with the tissue for 30 min before repeating the 5-HT concentration-response curves in the continued presence of the same concentration of antagonist. *c*, Concentration-response curves for the contractile action of 5-HT in the presence and absence of compound III were conducted in the guinea pig ileum incubated with 10^{-6} M methysergide as described previously¹⁰. The antagonist was permitted to equilibrate with the ileum for 30 min before repeating the concentration-response curves in the continued presence of the same concentration of antagonist.



relevant effects in either system. Thus, 5-HT₂ receptors in rat cortex are different from M-receptors found on peripheral neurones, although they may be similar to D-receptors found in the uterus and other smooth muscle preparations²¹.

M-receptor antagonists

Our results show that 2-methyl-5-HT and *S*(+)- α -methyl-5-HT are selective M- and D-receptor agonists, respectively. We next attempted to synthesize analogues of 5-HT that retained their affinity and selectivity for M-receptors but that lacked intrinsic activity; in other words, compounds that could occupy these receptors without activating them. Such compounds should behave as competitive antagonists of 5-HT at M- but not D-receptors. By extending the ethylamine chain of 5-HT and making rigid analogues where the terminal nitrogen was included in a tropine or homotropine ring system (see Table 2), we obtained a large series of competitive M-receptor antagonists, with remarkably high affinity and selectivity. Such compounds behave as true competitive 5-HT antagonists on all three M-receptor systems (Fig. 2; Table 2). However, the pA_2 values obtained for compound I (3- α -homotropanyl)-1-methyl-5-fluoroindole-3-carboxylic acid ester) or those for compound II (*N*-desmethyl-3- α -homotropanyl)-1*H*-indole-3-carboxylic acid ester) differed significantly from tissue to tissue ($P < 0.01$, paired *t*-test), indicating that the M-receptors in the vagus, heart and ileum are not identical. Thus, compound I is a highly selective antagonist for the type of M-receptor found in the vagus nerve, whereas compound II shows selectivity for that found in the heart. Both compounds yielded significantly lower pA_2 values for the M-receptors in the guinea pig ileum than for those in

either the vagus or heart, showing the presence of a third type of M-receptor in this tissue. The conclusion that the M-receptors in the ileum and heart differ from one another is in agreement with that of earlier studies where (-)norcocaine also yielded significantly different pA_2 values in these tissues¹¹. None of the M-antagonists so far synthesized has shown appreciable affinity for uterine D-receptors or for 5-HT₁ or 5-HT₂ receptors in the rat cortex ($pK_D < 5.0$ in all three systems, $n = 3$).

As M-antagonists became available, we also tested the selective M-receptor agonist, 2-methyl-5-HT, against them to check that the effects of this agonist in the vagus, heart and ileum were the consequence of M-receptor activation. As shown in Table 2, the pA_2 values obtained were not statistically different ($P > 0.05$, paired *t*-test) from those using 5-HT as agonist, showing that both agonists stimulate a common receptor in each tissue, despite the fact that a different subset of these receptors is found in each of the three tissues, as indicated above. Thus, both 5-HT and 2-methyl-5-HT have full agonist action at each of the three M-receptor subtypes.

The third antagonist listed in Table 2, (3- α -tropanyl)-1*H*-indole-3-carboxylic acid ester, termed ICS 205-930, is a compound with similar affinity for both the M-receptors in the vagus nerve and for those in the heart, but again shows lower affinity on the ileum. This compound has negligible affinity for uterine D-receptors or for 5-HT₁ or 5-HT₂ receptors in the rat cortex. In a series of ligand binding assays it was also demonstrated that this compound lacks affinity ($pK_D < 6.0$) for α_1 , α_2 , β_1 or β_2 adrenoreceptors, D-1 or D-2 dopamine receptors, H₁ histamine receptors, muscarinic receptors or benzodiazepine receptors (data not shown). This makes ICS 205-930 a potent and selective 5-HT M-receptor antagonist.

Actions of M-receptor antagonists

A bolus injection of 5-HT into the jugular vein of anaesthetized rats induces a vagally mediated reflex bradycardia²². This effect, the von Bezold-Jarisch reflex, can be elicited in several species by various compounds which stimulate sensory nerve endings located in the wall of the right ventricle²³. Recent studies have shown that both metoclopramide and several cocaine analogues can block the 5-HT-induced von Bezold-Jarisch reflex because, in addition to their various other pharmacological properties, they act as neuronal 5-HT receptor antagonists^{24,25}. Consistent with the mediation of this 5-HT effect being through M-receptor activation is the finding that the reflex can also be elicited by the selective M-receptor agonist 2-methyl-5-HT at doses as low as 5 µg per kg (see Fig. 3a), but not by the selective D-receptor agonist *S*(+)-α-methyl-5-HT at doses as high as 1,000 µg kg⁻¹ (*n* = 5), and that the ability of 5-HT to induce the von Bezold-Jarisch reflex is not prevented by pretreatment of the rats with the D-receptor antagonist methysergide (Fig. 3b). In contrast, an intravenous dose of 1 µg per kg of the selective M-receptor antagonist ICS 205-930 caused a parallel rightward shift of the 5-HT dose-response curve, such that a fourfold increase in the dose of 5-HT was necessary to produce the same reflex reduction in heart rate as occurred before administration of the antagonist (Fig. 3b). Thus, M-receptors are responsible for the initiation of this reflex by 5-HT and can be blocked by very low doses of ICS 205-930.

Inhibition of 5-HT-induced pain

5-HT causes pain when applied to a blister base on the human forearm and it markedly potentiates the vascular pain produced by bradykinin^{26,27}. Therefore, we investigated whether these actions are mediated by D- or M-receptors.

The M-receptor agonist 2-methyl-5-HT was as effective as 5-HT in inducing a pain response, whereas the D-receptor agonist *S*(+)-α-methyl-5-HT was ~100 times less potent (Fig. 4). Consistent with the pain-producing effects of 5-HT being mediated through M-receptor activation was the finding that the D-receptor antagonist methysergide did not inhibit 5-HT-induced pain, whereas ICS 205-930 at the low concentration of 10⁻⁸ M caused a marked rightward shift of the 5-HT pain dose-response curve. The inhibitory effect of ICS 205-930 could be

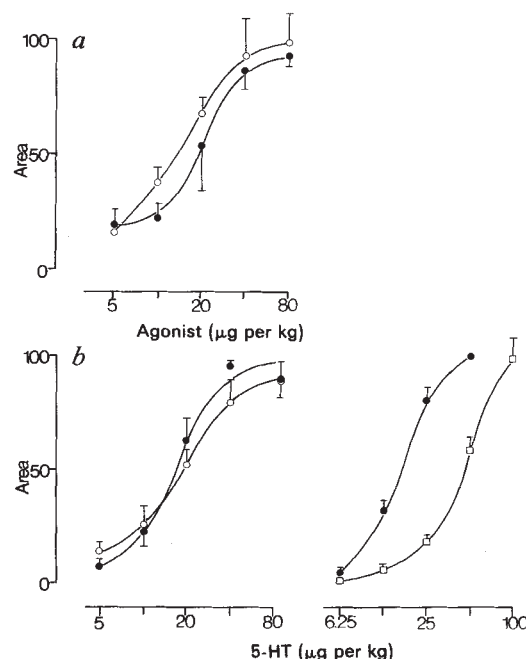


Fig. 3 The von Bezold-Jarisch effect in anaesthetized rats. *a*, Dose-response curves for 5-HT (●; *n* = 5) and 2-methyl-5-HT (○; *n* = 4). The selective D-agonist *S*(+)-α-methyl-5-HT was totally inactive up to a dose of 1,000 µg per kg, the highest one tested (*n* = 5, data not shown). *b*, Dose-response curves to 5-HT before (●; *n* = 5) and 5 min after the intravenous administration of the 5-HT D-receptor antagonist methysergide (○; 100 µg per kg; left panel; *n* = 4) or the M-receptor antagonist ICS 205-930 (□; 1 µg per kg; right panel; *n* = 5). Vertical bars indicate s.e.m. in each case. 'Area' refers to the area under the time-heart rate curve. **Methods.** Male rats weighing 220–270 g were anaesthetized by injecting urethane (1.25 g per kg) into the peritoneal cavity. Blood pressure and heart rate were recorded by standard techniques using a Satham transducer. Injections were made into the jugular vein. Rapid bolus injections of 5-HT (5–100 µg per kg) given at 5-min intervals elicited abrupt dose-related reductions in cardiac rate of short duration and reproducible magnitude. The area under the curve describing the reflex bradycardia (ordinates) quantified the effect.

Table 2 Antagonism of M-receptors in isolated tissues

Antagonist	Tissue	Agonist	pA_2 value ± s.e.m.	Slope of $\log(DR - 1)$ on $\log(B) \pm$ s.e.m.
<chem>CN1[C@H]2CC[C@@H]1C(=O)Nc3cc(F)ccc3</chem> Compound I	Vagus	5-HT	13.10 ± 0.78	0.76 ± 0.21
		2-Methyl-5-HT	13.55 ± 0.77	0.70 ± 0.19
	Heart	5-HT	10.11 ± 0.09	1.17 ± 0.29
	Ileum	5-HT	9.10 ± 0.13	0.91 ± 0.15
<chem>CN1[C@H]2CC[C@@H]1C(=O)Nc3ccccc3</chem> Compound II	Vagus	5-HT	8.90 ± 0.18	0.82 ± 0.12
		5-HT	9.83 ± 0.14	1.26 ± 0.27
	Heart	2-Methyl-5-HT	9.70 ± 0.05	1.16 ± 0.24
	Ileum	5-HT	7.74 ± 0.22	0.98 ± 0.17
<chem>CN1[C@H]2CC[C@@H]1C(=O)Nc3ccccc3</chem> Compound III (ICS 205-930)	Vagus	5-HT	10.23 ± 0.19	0.95 ± 0.18
		5-HT	10.63 ± 0.22	0.92 ± 0.46
	Heart	5-HT	7.85 ± 0.10	1.22 ± 0.19
	Ileum	2-Methyl-5-HT	8.03 ± 0.05	0.93 ± 0.03

Experiments to assess the affinities of three competitive M-receptor antagonists against 5-HT or 2-methyl-5-HT on the three isolated tissues were performed as described in Fig. 2 legend. The pA_2 and slope values were obtained by transforming the data from concentration-response curves to 5-HT or 2-methyl-5-HT conducted in the absence or presence of the respective antagonist from a minimum of three independent experiments, as described elsewhere³⁵. DR , dose ratio (the factor by which the agonist concentration has to be increased in the presence of the antagonist to obtain a response identical in magnitude to that recorded in the absence of the antagonist); B , antagonist concentration. If 5-HT and the antagonist under consideration compete for a common site, then $\log(DR - 1) = \log(B) - \log K_B$, where K_B is the apparent dissociation constant for the antagonist-receptor interaction³⁶. In this case the regression of $\log(DR - 1)$ on $\log(B)$ (Schild plot³⁵) yields a straight line of unity slope and a pA_2 value of $-\log K_B$. Slope and pA_2 values given here were obtained from linear regression analysis of the Schild plots. In all three tissues, compounds I, II and III behaved as true competitive antagonists, causing parallel rightward displacements of the agonist concentration-response curves without significantly reducing the maximum effect. In addition, the slope of $\log(DR - 1)$ on $\log(B)$ was not significantly different from unity (*t*-test), showing that 5-HT and the antagonists compete for common receptor sites in these tissues.

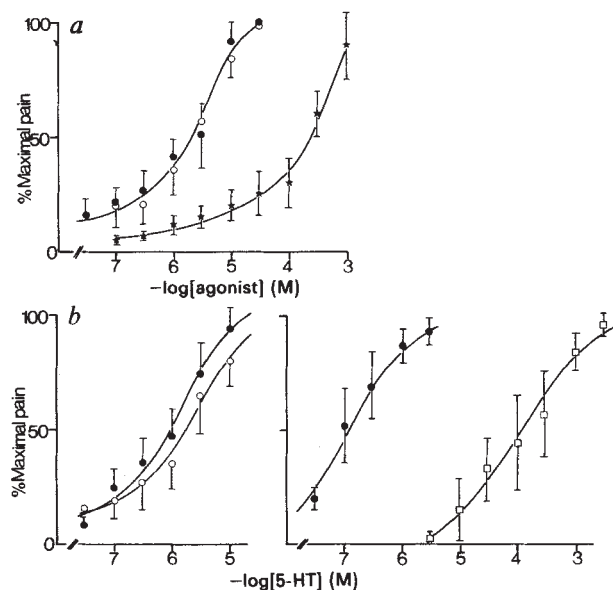


Fig. 4 Painful effects of 5-HT and methylated analogues when applied to a blister base on the human forearm. *a*, Effects of 5-HT ($\bullet$; $n=5$), 2-methyl-5-HT ($\circ$; $n=3$) and S(+)- α -methyl-5-HT ($\bullet$; $n=3$) in causing pain. *b*, Concentration-response curves for 5-HT in the absence ($\bullet$; $n=4$) or presence of 10^{-5} M methysergide ($\circ$; $n=3$; left panel) or 10^{-8} M ICS 205-930 ($\square$; $n=4$; right panel). Antagonists were applied to the blister base for 20 min before applying 5-HT in their continued presence. Vertical bars indicate s.e.m. in each case.

Methods. Double-blind studies were conducted on five healthy male volunteers aged 32–55 yr. Blisters were produced on the medial aspect of the forearm as described previously²⁶. Increasing concentrations of 5-HT (10^{-8} – 10^{-5} M) were applied to the blister base in Krebs-Ringer solution. The contact time in each case was 2 min. Subjects were asked to register the pain experienced during each 2-min interval by moving a lever graduated on a 0–3 rating scale (0 = no pain, 1 = slight pain, 2 = moderate pain, 3 = severe pain) coupled to a linear integrator that then recorded the area under the 'pain intensity-time curve'. The concentration of 5-HT was increased every 2 min until one was reached where no further pain was produced. In this way, a cumulative dose-response curve for 5-HT could be constructed.

fully reversed on washing off the antagonist with buffer for 60 min.

To confirm that this effect of ICS 205-930 reflected a specific antagonism of 5-HT and not a general time-dependent reduction in the sensitivity of the blister base to all painful stimuli, dose-response curves for bradykinin (10^{-8} – 10^{-6} g ml⁻¹) were performed before, during and after incubation of the blister with ICS 205-930. The three dose-response curves were virtually superimposed and the maximum response similar at each time point (data not shown). Thus, in contrast to its effect on 5-HT responses, ICS 205-930 did not inhibit bradykinin-induced pain.

Concomitant with the pain response, 5-HT caused a wheal and flare reaction that presumably occurred as a result of the activation of sensory nerve endings in the skin and the consequent liberation of substance P by an axonal reflex mechanism²⁸. This was also inhibited by 10^{-8} M ICS 205-930. At the higher concentrations of 5-HT needed to induce pain in the presence of the antagonist, the wheal and flare response reappeared.

5-HT also caused a marked potentiation of bradykinin pain responses and this could be abolished by ICS 205-930 (Fig. 5). This inhibitory effect was partly reversed on washing off the antagonist for 20 min. Thus, ICS 205-930 not only inhibits pain responses to 5-HT in this system, but also blocks its ability to potentiate bradykinin responses.

These studies show that 5-HT induces its painful effect in humans through activation of neuronal M-receptors and that this can be selectively and reversibly inhibited by the M-receptor antagonist ICS 205-930.

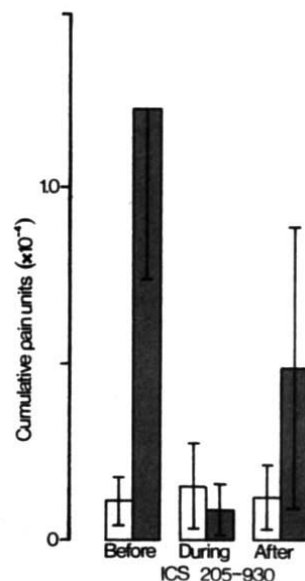


Fig. 5 Potentiation of the painful effects of bradykinin on the human blister base by 5-HT and inhibition of this effect by ICS 205-930. Pain produced by a solution containing 10^{-8} g ml⁻¹ bradykinin alone (open columns) or in combination with 10^{-4} M 5-HT (hatched columns) was recorded before, during and after incubation of the blister base with 10^{-8} M ICS 205-930. Mean values obtained from five male volunteers are given. Vertical lines on each column indicate s.e.m.

Methods. Double-blind studies were performed in a further group of male volunteers aged 22–45 yr as described in Fig. 4 legend. In this series of experiments 10^{-8} g ml⁻¹ bradykinin was applied to the blister base first and the response recorded. This concentration of bradykinin produced a small pain response ('before', open column). It was subsequently washed off and the blister base incubated with buffer for 20 min before 10^{-4} M 5-HT was applied repeatedly. Initially this induced a large pain response as expected, but this subsided over the next 2–6 min despite repeated reapplication of the same 5-HT concentration. Subsequently 10^{-8} g ml⁻¹ bradykinin was applied to the blister in the continued presence of 10^{-4} M 5-HT for 2 min, resulting in a 10-fold increase in the bradykinin response ('before', hatched column). The bradykinin/5-HT combination was then washed off for 20 min with buffer, after which the blister base was incubated with 10^{-8} M ICS 205-930 for a further 20-min period. The responses to bradykinin (10^{-8} g ml⁻¹; 'during', open column) and the bradykinin/5-HT combination (10^{-8} g ml⁻¹ and 10^{-4} M, respectively; 'during', hatched column) were then recorded in the continued presence of 10^{-8} M ICS 205-930. ICS 205-930 completely inhibited the potentiation of bradykinin pain caused by 5-HT. This inhibitory effect of ICS 205-930 was partly reversed on washing off the antagonist for 20 min ('after').

Discussion

Compounds such as the ergot alkaloids, which possess the ability to inhibit the action of 5-HT at D-receptors on smooth muscle cells or at postsynaptic 5-HT₂ receptors in the central nervous system, have been available for many years²⁹. Here we describe the synthesis of highly potent and selective antagonists of another class of 5-HT receptors, M-receptors, located on peripheral nerves. Significant differences in the affinity constants obtained with the same competitive antagonist on different bioassay systems indicate that there are at least three distinct subclasses of these M-receptors.

During the course of this work we became aware of a similar project by J. Fozard and his colleagues. Based on earlier observations that (–) cocaine and structurally related compounds act as competitive antagonists at neuronal 5-HT receptors in the rabbit heart and guinea pig ileum^{8,10,11}, this group synthesized analogues with improved potency and selectivity, which yielded a series of compounds that are antagonists at 5-HT M-receptors in the heart³⁰. A report describing the pharmacological properties of one of these drugs, MDL 72222 (3,5-dichloroben-

zoxytropine ester), appeared during the preparation of this manuscript³¹. Unlike the indoletropanyl and homotropanyl esters synthesized by our group, none of these benzoyltropine analogues blocks 5-HT M-receptors in the guinea pig ileum or exhibits the remarkably high potency for vagal M-receptors achieved with many of our antagonists¹⁸. Indeed, some of the indole compounds we have synthesized are the most potent drugs of any pharmacological class so far described.

Studies in humans and rats with the selective antagonist ICS 205-930 show that M-receptors are present on sensory nerve endings in the skin and right ventricle of the heart. The ability

of M-receptor antagonists to block selectively the painful effects of 5-HT when applied to a blister base on the human forearm suggests that such compounds might present a novel approach to the management of conditions such as migraine and cluster headaches, where 5-HT may be involved in the genesis of vascular pain^{27,32}. This possibility has been discussed previously in detail²⁴ and encouraging preliminary clinical results with MDL 72222 support this contention³³.

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LETTERS TO NATURE

Statistical significance of the 59-s periodicity of Geminga and 1E0630+178

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Bignami *et al.*¹ have reported evidence for an increasing 59-s period in the X-ray emission from the high-energy ($E \geq 50$ MeV) γ -ray source Geminga. Their claim is based on results of a search for periodicities at around 59s in the emission from the X-ray source 1E0630+178, which has been proposed as a possible counterpart of Geminga². We show here that the primitive indication by SAS-2 (ref. 3) of a 59-s periodicity in the γ -ray flux from Geminga is not statistically significant, and because it has also not been confirmed by COS-B (ref. 4), must be disregarded; the statistical significance of the results obtained from periodicity searches in the X-ray data is significantly lower than that reported by Bignami *et al.*¹. As a consequence, the identification of 1E0630+178 with the γ -ray source 2CG195+04 (Geminga) through a 59-s pulsation cannot be claimed.

The first suggestion of a possible 59-s periodicity in the Geminga γ -ray emission was derived from SAS-2 data³. The analysis was performed on a data set consisting of 121 photons, energy > 35 MeV, detected over three separate observation intervals, each about 1 week long, with 37, 28 and 56 counts respectively. These data were searched for pulsation and the result was a 25-bin phase histogram with a χ^2 of 78 for a trial period of 59.0074s, increasing at a constant rate of 2.23×10^{-9} s per s. Without entering into the details of the analysis method used by Thompson *et al.*³, we can evaluate the chance occurrence probability of that result. For 121 counts uniformly spread over 25 bins the central limit theorem does not hold and the theoretic-

cal probability distribution of the χ^2 cannot be used. We derived the actual probability distribution by making a Monte-Carlo simulation of 10^6 sets of 121 counts uniformly distributed in three observation intervals as in the SAS-2 experimental conditions. The result is shown in Fig. 1. It is evident that $P(\chi^2 \geq 78) \approx 1 \times 10^{-6}$. Therefore, no more than 1,000 trials are allowed in order for the SAS-2 indication to be statistically significant. The number of trials used by the SAS-2 team was actually 8×10^4 around an input period of 58s (D. Thompson, personal communication), which implies a global chance occurrence probability of $\sim 8\%$ for the reported effect. Furthermore, as the choice of the input period was not derived from independent studies but from an investigation of the same γ -ray data, the implicit number of trials made to identify it should also be taken into account. Therefore, the value of 8% is a lower limit. On the other hand, Thompson *et al.*³ were aware of the weakness of their indication. After showing the effect they, in fact, declared that "... in view of the number of trials used ... the evidence for this periodicity is not statistically compelling but is worth examination by future experiments".

COS-B has observed the general direction of Geminga five times. Bignami *et al.*¹ refer to the results of a preliminary analysis of the first observation, made from 17 August to 17 September 1975, during which COS-B was pointing at the Crab pulsar and Geminga was visible at 15° off axis⁵. The quoted Caravane Collaboration paper stated a confirmation (1% chance occurrence probability) of the 59-s effect claimed by SAS-2. However, the χ^2 of the histogram, obtained after 133 trials was 43.6 and then, in our reevaluation, the global chance occurrence probability of the COS-B effect is $1.2 \times 10^{-3} \times 133 \approx 16\%$. Later, the COS-B team extended the analysis when the data from two more observations became available. Due to an increase in source counts by a factor of ~ 5 and more optimized selections, a much better signal-to-noise ratio than in the previous analysis became available. As a result, Masnou *et al.*⁴ concluded that "... no periodicity is observed around the 59s value". Note that the γ -ray flux of Geminga has remained constant within the statistical uncertainties from the SAS-2 (ref. 3) observations to those of COS-B (ref. 6). Therefore, applying the reasonable

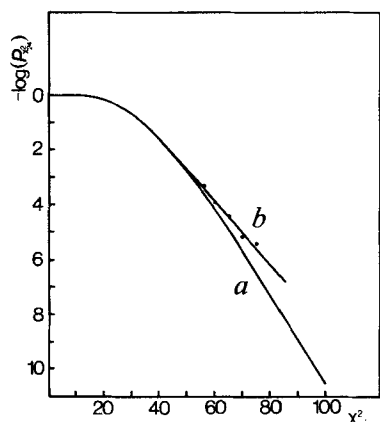


Fig. 1 *a*, Theoretical probability distribution of the χ^2 with 24 d.f. *b*, Probability distribution of the variable $y = \sum (x_i - \bar{x})^2 / \bar{x}$ where $\bar{x} = 121/25$ and x_i is the content of the i th bin of the histogram obtained by folding, with a period of 59s, the arrival times of 121 events, uniformly distributed in three time intervals of 7, 5 and 7 days, respectively (total elapsed time between the beginning of the first week and the end of the third week is 131 days, as in the SAS-2 case).

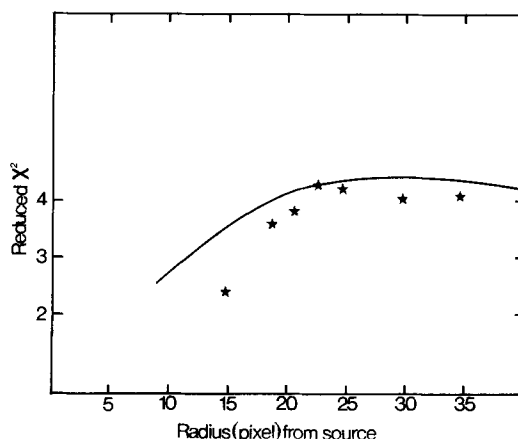


Fig. 2 Distribution of the expected reduced χ^2 as a function of radius R (solid line) compared with reduced χ^2 values ($\star$) obtained in ref. 1 by folding the IPC 10371 data with a period of 59.737s.

hypothesis that the shape of the light curve and the pulsed fraction of the flux has also remained stable, we expect a strong signal in the COS-B data due to the high counting statistics. In fact, the peak of the SAS-2 light curve when scaled to the COS-B exposure of Geminga would show up at the level of at least 16σ in the case of observation period 14 alone. Despite all the effort devoted by the Caravane Collaboration to detect periodicity effects in the Geminga data, no such result has been found.

The absence of a timing signature in the γ -ray emission from Geminga makes it impossible to identify a counterpart at other wavelengths. This also applies to the candidate-counterpart X-ray source 1E0630+178. Nevertheless, Bignami *et al.*¹ have centred the temporal analysis of the X-ray data of 1E0630+178 at the disclaimed Geminga period of 59s. The reported X-ray time analysis can also be criticized for several reasons:

(1) The chance occurrence probability of the 59-s effect in the Einstein 10371 data has been incorrectly calculated. As a result of the 20 trial periods scanned, a reduced χ^2 of 3.94 was obtained, corresponding to a global chance occurrence probability of $\sim 5 \times 10^{-5} \times 20 = 1 \times 10^{-3}$. Bignami *et al.*¹ quote a probability of 2×10^{-5} for the same data. This error is due to two reasons. First the single trial probability used to obtain a reduced χ^2 of 4.26 (9 d.f.) was 1.5×10^{-5} (ref. 7) and not 1×10^{-6} . Second, the value of 4.26, used instead of 3.94, is the result of fine scanning performed in addition to the initial 20 trials. Therefore, the appropriate number of periods searched is higher than 20, the value used by Bignami *et al.*¹. The same criticism applies to the combined data of IPC 10371 and HRI 8353, again an error in the single trial probability and the underestimated number of trials.

(2) Bignami *et al.*¹ fixed, *a priori*, the radius R (in pixels of 8 arc s) from the source position at $R = 23$ in their event selection and, correctly, discuss, *a posteriori*, whether the distribution of χ^2 values as a function of R behave as expected for a genuine source effect. This can be better judged from Fig. 2 which shows the reduced χ^2 expected for various values of R as derived from the relation $\text{reduced } \chi^2 = C \times NS^2 / NT + 1$ (ref. 8) (where NS is the number of source counts and NT is the total number of counts taken from Fig. 1 of ref. 1 and C is obtained by normalization at $R = 23$). Figure 2 shows that the χ^2 value at $R = 23$ is an upward fluctuation from the expected general trend. Therefore, the probability level of 1×10^{-3} is the lower limit to the probability that the reported effect is due to a random fluctuation.

(3) The useful observation time of the first Einstein observation (IPC 3333) is only 0.29 of that of the second and we expect a reduced χ^2 of 2.1 (chance occurrence probability of 50% in 20 trials). The measured value of 3.6 can only be reconciled with the IPC 10371 result and the SAS-2 indication, on which this search has been based, if three parameters are adjusted, namely the P value, the intensity of the pulsed emission from 1E0630+178, and possibly the shape of its light curve. Therefore, the effect found in the IPC 3333 data cannot be considered as independent evidence of a genuine effect, nor can the significances derived from the two observations be combined without taking into account the additional degrees of freedom.

Finally, we dispute the arguments based on the Zyskin and Mukanov results⁹ derived from measurements at ultra-high energies in 1979 and 1981. First, the result derived from the 1979 observations is declared by the authors to be statistically weak (6% chance occurrence probability). Second, for the 1981 data Zyskin and Mukanov show their 'light curve' with 19 bins and a χ^2 of 43.6. To obtain this effect as a random fluctuation, they use 10 d.f. in their probability calculation instead of 18. Using 18 d.f. the probability increases up to $6 \times 10^{-4} \times 100 = 6 \times 10^{-2}$ (compared with their quoted probability of 5×10^{-4}). Therefore, the result of the analysis of the 1981 data cannot be considered significant either. In addition, the obtained period is not consistent with the P and $\dot{P}$ derived from the Einstein or SAS-2 data and cannot be taken as independent evidence.

We conclude that the identification of Geminga with 1E0630+178 and the consequent speculations made by Bignami *et al.* are based on arguments too weak to be conclusive. The mystery of 2CG195+04 does not seem to be solved, although 1E0630+178 continues to be an interesting candidate counterpart.

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Joint STARE and SABRE radar auroral observations of the high-latitude ionospheric convection pattern

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The projection of the magnetospheric convection electric field into the ionosphere drives an asymmetric two-cell pattern of plasma circulation in the auroral zone. The Scandinavian Twin Auroral Radar Experiment (STARE) and the Sweden And Britain auroral Radar Experiment (SABRE) are two similar and independent coherent radar systems which can estimate *E*-region electron drift velocities at auroral latitudes. By combining their measurements, we have monitored simultaneously, for the first time, the convection flow pattern over 8 degrees of latitude. We show here how this allows a more extensive study of the nightside (Harang discontinuity) and dayside (cusp) boundaries between the cells, and enables spatial and temporal features of the flow pattern to be resolved.

The STARE and SABRE systems consist of two multi-beam radars that are sensitive to electrostatic ion-acoustic waves, which are produced in association with current flows in the auroral zone E-layer. The phase velocity of these irregularities is approximately equal to the electron drift velocity¹, and can be determined from the mean Doppler shift of the transmitted radar signal. Each radar measures the backscatter intensity and Doppler velocity of irregularities at each of 50 ranges within eight beams, providing a total common viewing area in the auroral E-region of ~200,000 km². The spatial resolution is ~20×20 km and the temporal resolution is usually 20 s. The Doppler shifts measured by the two radars are combined to give the electron drift velocity in a two-dimensional array of geographical locations within the viewing area. Recent EISCAT/STARE comparisons² indicate that the coherent auroral radar underestimates the higher flow speeds, although the directions are accurate. An empirical correction factor has been developed, the validity of which is yet to be established for SABRE. No correction has therefore been applied to the data presented here.

The STARE system³ has been operated by the Max-Planck-Institut für Aeronomie (MPI), Lindau, FRG, since 1977 and consists of radars at Malvik in Norway and Hankasalmi in Finland. SABRE⁴ was completed in 1982 and is operated jointly by Leicester University, UK and by MPI. The two SABRE radars are located at Wick in Scotland, and at Uppsala in Sweden.

In 1961, Axford and Hines⁵ proposed that viscous interaction between the solar wind and a closed magnetosphere would produce a high-latitude two-cell ionospheric convection pattern. At the same time, Dungey⁶ introduced the concept of solar wind interaction with an open magnetosphere through magnetic reconnection. This mechanism involves the merging of geomagnetic field lines on the dayside of the Earth's magnetopause with the interplanetary magnetic field (IMF). As the IMF is frozen into the solar wind, the opened field lines are 'dragged' across the poles, transporting plasma in an anti-sunward direction. The field lines reconnect in the magnetospheric tail and the return flow is on closed field lines around the flanks of the magnetosphere. It is generally accepted that both viscous interaction and magnetic reconnection processes drive plasma convection within the magnetosphere and that the ionospheric projection of these motions takes the form of a two-cell pattern (Fig. 1). Although the gross features of this pattern have now been well established, recent observations^{7,8} indicate that the shape and relative sizes of the convection cells are considerably modified by geomagnetic substorms and by the orientation of

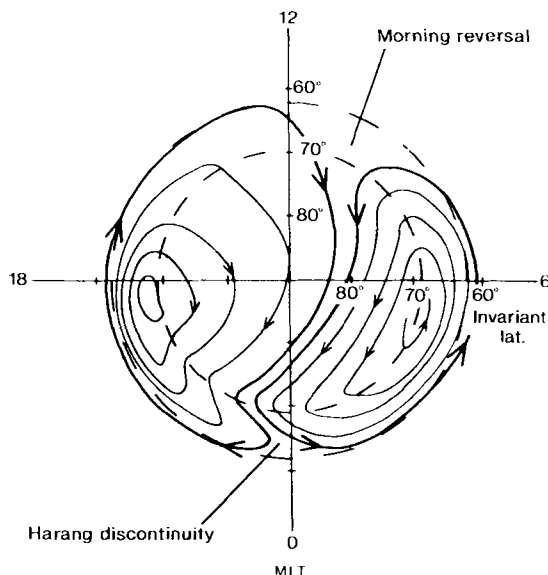


Fig. 1 The two-cell convection pattern, plotted in invariant latitude against magnetic local time (MLT), for moderately disturbed geomagnetic conditions. Superimposed is the path taken by the combined STARE and SABRE viewing area, as the Earth rotates beneath the cell pattern.

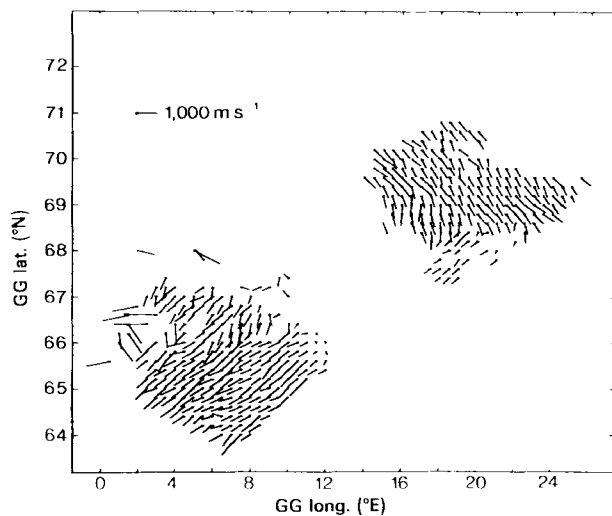


Fig. 2 Simultaneous STARE and SABRE flow velocity observations made at 1911 UT 6 September 1982 in the Harang discontinuity region. The velocities are given as a function of latitude and longitude.

the IMF. From several days of STARE observations, it has been suggested⁹ that as magnetic activity increases: (1) the convection flow speeds increase; (2) the flow pattern expands; (3) the morning cell is enlarged with respect to the evening cell; and (4) the whole convection pattern rotates clockwise (in the Northern Hemisphere) towards earlier local times.

We now consider simultaneous STARE and SABRE observations of flows associated with both the morning and the evening convection reversals. An example of the convection flows measured in the vicinity of the evening reversal or Harang discontinuity is shown in Fig. 2 which indicates the convection flow vectors at 1911 UT 6 September 1982 in both viewing areas, with the SABRE field of view located to the south-west. The length and direction of each vector represent the flow speed and direction respectively. The flow direction in the STARE field of view is predominantly eastward, while that in the SABRE viewing area is westward. From an examination of the temporal development of the flow pattern, it was established that at this

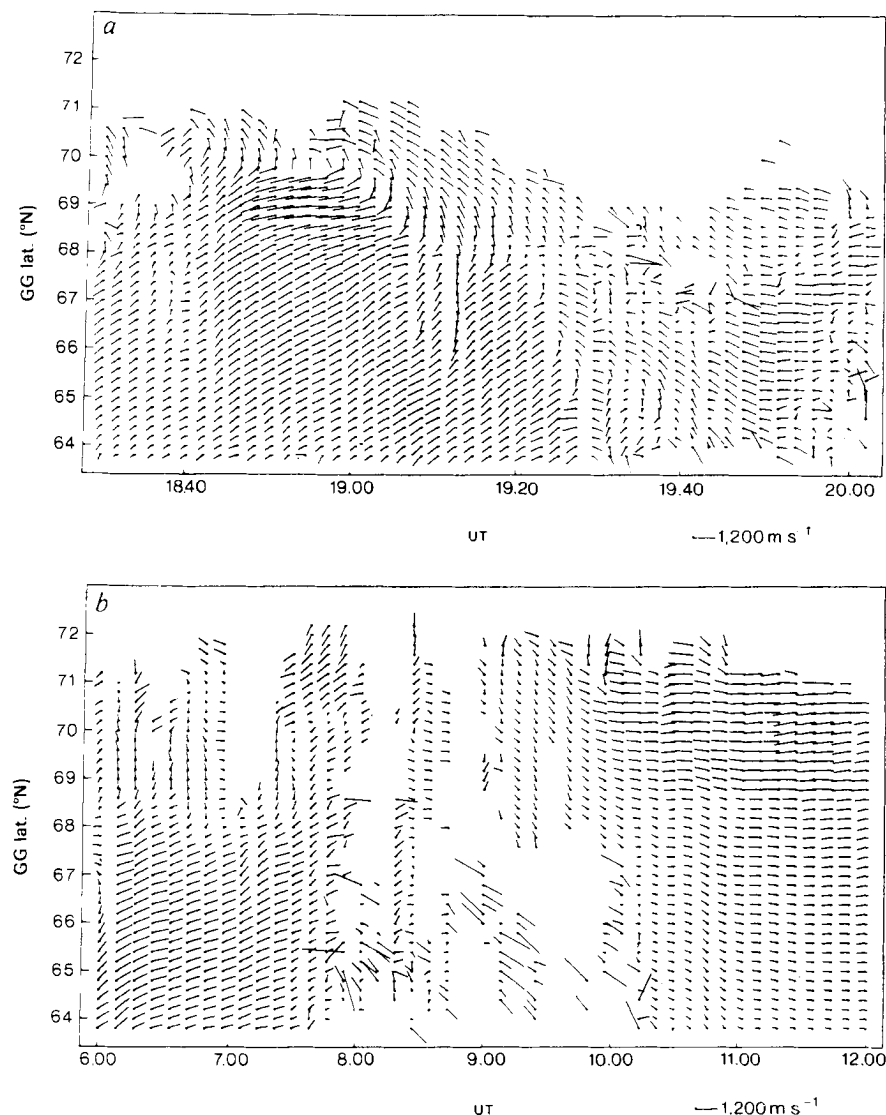


Fig. 3 Combined STARE and SABRE convective flow velocity measurements for *a*, 6 September 1982, 1830–2000 UT; *b*, 7 August 1982, 0600–1200 UT. The velocities are given as a function of latitude and time, averaged over the longitude range 8–18° E.

time the viewing areas lie either side of the Harang discontinuity, with STARE and SABRE observing the morning and evening convection cells respectively. Figure 3*a* illustrates the convection flow velocities, as a function of latitude and time, averaged over the longitudinal interval 8–18° E. This is chosen to include both the eastern edge of the SABRE viewing area and the western edge of the STARE viewing area. Because of the longitudinal displacement between the two viewing areas, difficulties arise with this averaging process when the convection flow is not spatially uniform. The plot covers the period 1830–2000 UT, during which time the Harang discontinuity passed through both viewing areas. Figure 3*a* indicates the transition from the region of westward flow, which is associated with the evening convection cell, to that of eastward flow associated with the morning cell. The discontinuity moves equatorward with time throughout the entire latitude range, although the penetration speed of the eastward flow is latitudinally dependent. The equatorward drift is $\sim 250 \text{ m s}^{-1}$ within the STARE viewing region but has increased to $1,000 \text{ m s}^{-1}$ by the time the discontinuity passes through the SABRE viewing area. The sudden change at around 68° N arises from the longitudinal resolution limitation mentioned above, and indicates the presence of strong spatial velocity variations in the auroral E-region. Owing to the exceptionally high level of magnetic activity at this time ($K_p = 7$), the convection cell pattern has expanded equatorwards, and the strong auroral zone flows normally found at latitudes above 67° N are located within the latitude range covered by SABRE. The STARE radar measures weaker flows characteristic of the

polar cap region. In quiet geomagnetic conditions, the Harang discontinuity would be expected to occur at 2300 UT (refs 7, 9). This particular reversal took place between 1850 and 1940 UT, implying a clockwise rotation of the entire cell pattern by ~ 3 –4 h. Despite the magnetically-active conditions, the gross features of the observed flows are in good agreement between the two systems, demonstrating that STARE and SABRE may be usefully combined for increased latitudinal coverage in the morphological study of convection flows in the auroral ionosphere.

Although the Harang discontinuity is frequently observed by STARE and SABRE, convection flows associated with the morning reversal are very weak at these latitudes. In highly disturbed conditions, however, strong flows can occur in this region at latitudes below 64° N (refs 8, 10). Figure 3*b* is an example of a morning sector observation, illustrating the temporal development of the plasma flow pattern in the vicinity of the convection reversal, for the time interval 0600–1200 UT 7 August 1982. The value of K_p was +7 during the period 0600–0900 UT and –7 between 0900 and 1200 UT. In contrast to the evening sector example, the change in flow direction is from eastwards to westwards, and the flow pattern displays a throat-like structure which widens with decreasing latitude. Although auroral radar observations of the morning reversal are rare, they are of particular interest because in this local time sector the Earth's field lines map directly into the outer magnetosphere. Measurements made in this region of the high-latitude ionosphere can, therefore, be used to infer the properties of the plasma in the polar cusp, which is the highly dynamic zone of direct interaction

between the Earth and the interplanetary environment. Future studies involving the correlation of STARE and SABRE cusp flow observations with the behaviour of the IMF will enhance our understanding of the field line reconnection mechanism by which the solar wind drives the high-latitude convection cell pattern.

The STARE radar was constructed and is operated by MPI, Lindau, FRG. SABRE was constructed and is operated jointly by MPI and the Department of Physics, Leicester University, UK.

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Increasing abundance of CBrClF₂ in the atmosphere

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The importance of bromine chemistry was recognized shortly after Molina and Rowland¹ had drawn attention to chlorine atom-catalysed destruction of ozone. Bromine atoms are even more efficient catalysts than chlorine^{2,3}, and both may act synergistically to deplete ozone in the lower stratosphere⁴. Some measurements of bromine-containing source gases, such as CH₃Br, CHBr₃, CH₂BrCH₂Br, CH₂Br₂, CBrF₃ and CBrClF₂ have been reported for the troposphere⁵⁻⁸, their vertical distribution in the stratosphere is unknown except for CBrF₃ (CFC-13B1) and CH₃Br (ref. 6). These, together with the fully halogenated CBrClF₂ (CFC-12B1), are probably the most important source gases for stratospheric bromine radicals. We report here the first stratospheric profiles of CBrClF₂ up to 25 km, measured at 44° N during three consecutive years. From these data it follows that the atmospheric abundance of this halocarbon is growing rapidly, at a rate of 20% per year in the Northern Hemisphere, corresponding to a worldwide emission of $\sim 3.6 \times 10^3$ tons per year.

Like CFC-13B1, whose vertical distribution has already been measured⁶, CFC-12B1 has an anthropogenic origin: both substances are used as fire extinguishers. Their ultraviolet absorption characteristics are similar to those of the corresponding chlorine-containing species, but shifted towards longer wavelengths. Thus halogen atoms are released from these molecules in the lower stratosphere, leading to overall atmospheric lifetimes of 29-42 yr and 62-117 yr for CFC-12B1 and CFC-13B1, respectively⁹. Both substances are abundant at ~ 1 p.p.t.v. (parts per 10^{12} by volume)^{5,8,10}. Compared with CH₃Br, the main natural bromine-containing halocarbon whose tropospheric mixing ratios were found to lie between 5 and 20 p.p.t.v. (refs 5, 8, 10), the anthropogenic CFC-12B1 and CFC-13B1 contribute between 10 and 30% to the present organically-bound bromine.

Stratospheric profiles of CBrClF₂ were obtained by analysing air samples collected cryogenically by means of neon-cooled samplers. The new MPAE samplers¹¹ allow the collection of samples of 30 litres STP (standard temperature and pressure) at 15 different altitudes up to 40 km. CBrClF₂, among other halocarbons, was analysed by gas chromatography with an electron capture (EC) detector. A 3 m $\times$ 2 mm internal diameter (i.d.) glass column packed with OV-101 on Chromosorb WHP

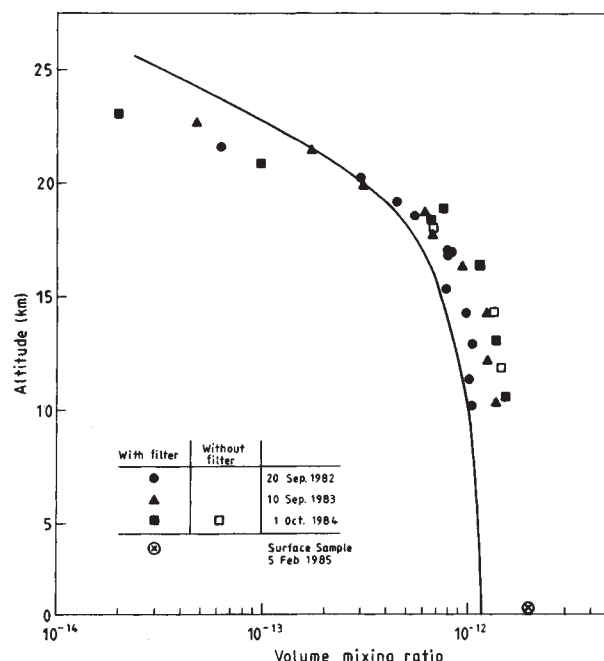


Fig. 1 The vertical distribution of CBrClF₂ (CFC-12B1) measured at 44°N during three consecutive years. The theoretical profile was computed by means of a time-dependent one-dimensional model, for 20 September 1982.

80-100 mesh was used for separation, with temperatures varying between -50 and +60 °C and argon/methane (10%) as the carrier gas¹². In combination with a cryotrap preconcentrator, CFC-12B1 is analysed with a precision of $\pm 5\%$, the detection limit being 0.03 p.p.t.v. The absolute calibration is achieved by two-step static dilution with $\sim \pm 10\%$ accuracy.

Results from three balloon flights carried out over southern France, on 20 September 1982, 10 September 1983, and 1 October 1984, are presented in Fig. 1. Most samples were collected with a copperwool filter attached to the air intake, to destroy incoming ozone¹³. Sampling tests performed in simulated stratospheric conditions, with gas mixtures containing 5 p.p.t.v. of CFC-12B1, gave no indication of sampling errors exceeding the analytical precision ranges of $\pm 5\%$. In contrast to some other halocarbons, CFC-12B1 does not change in the sampler when ozone is added; thus no systematic differences occur between samples collected with or without this filter. All analyses were completed within 2 months after each flight.

By repetitive analyses and cross-checks, it was found that the sensitivity of the analytical system had changed in 1984. Thus, a correction factor of (1.14 ± 0.04) had to be applied to the 1982 and 1983 profiles. No change of sensitivity was found between 1982 and 1983. The steplike structures observed in the profiles shown in Fig. 1 reflect real variations which were also observed in the profiles of other source gases analysed together with CFC-12B1. Such structures were found to be due to large-scale advection processes^{14,15}.

If the lowest two samples of each flight are considered to be representative of high-tropospheric conditions (in fact, the tropopause was between 11 and 12 km for all three flights), the abundance of CFC-12B1 increased from 1.03 p.p.t.v. measured on 20 September 1982, to 1.31 p.p.t.v. on 10 September 1983, and to 1.49 p.p.t.v. on 1 October 1984. These values correspond to relative growth rates of 28% per year for 1983 and 12% per year for 1984, or 20% per year averaged over both years. If the worldwide emission pattern of CFC-12B1 follows that of other anthropogenic substances, it is reasonable to assume that >90% of the emission occurs in the Northern Hemisphere. If no hemispheric mixing were to occur, the increase of the abundance of CFC-12B1 observed at northern midlatitudes would correspond to a global annual release rate of 2.6×10^3 tons per year.

However, as interhemispheric mixing occurs with a time constant of ~ 2 yr, the global release rate is likely to amount to $\sim 3.6 \times 10^3$ tons per year. Considering the error of $\pm 4\%$ related to the sensitivity correction of our analytical system, the range of this value may be $\pm 8\%$.

No values for the global production and emission rates of CFC-12B1 are available. For model studies, we have adopted an (arbitrary) scenario of exponential growth with global emission rates doubling every 18 yr. Scaling of these emission rates was performed such that the modelled profile, computed with a time-dependent one-dimensional model for 20 September 1982, matched the lower part of the observed profile (Fig. 1). Annual emission rates obtained in this way increased from ~ 1 kton per year in 1955 to 3.07, 3.23 and 3.34 kton per year for 1980, 1981 and 1982, respectively. Although the average annual emission rate of 3.6 kton per year derived from our measurements, for the 1983–84 period, seems to be consistent with this adopted emission scenario, the large relative increase of 20% per year observed for CFC-12B1 indicates that the doubling time of the annual worldwide emission rates is likely to be much shorter than 18 yr. The rapid growth of the atmospheric abundance of CFC-12B1 is also confirmed by near-surface measurements. While Rasmussen *et al.*⁸ report a value of 1.1 ± 0.2 p.p.t.v. measured at Barrow (Alaska) during 1983, a sample of fresh polar air collected on 5 February 1985 close to the MPAE site, yielded 2 p.p.t.v. of CFC-12B1. The abundances of CFC-11, CFC-12, CH_3CCl_3 and CCl_4 , which were analysed together with CFC-12B1, gave no evidence of local or regional pollution of this sample.

In the stratosphere, CFC-12B1 mixing ratios decrease by two orders of magnitude, to $\sim 10^{-14}$ at 25 km. The profile computed with the time-dependent model shows a slower fall-off with height, which reflects a well-known modelling problem related to source gases¹². Note that the rapid annual increase of CFC-

12B1 abundance is confined to heights below 20 km. In all three cases, radiosonde measurements from nearby meteorological stations revealed temperatures beginning to rise with height above 20 km. As stratospheric measurements of CO_2 indicate, substances from anthropogenic sources found above 20 km, in the height region of positive temperature gradient, have mostly penetrated through tropical upwelling, while the height region below 20 km is mostly governed by diffusion¹⁶. The related time lag for the 20–30-km layer with respect to the troposphere was found to be ~ 5 yr (ref. 15). This would confirm that the worldwide emission of CFC-12B1 into the atmosphere has been high in recent years.

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Transient electro-optic Raman scattering in liquid crystals

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We report here the first realization of time-resolved electro-optic Raman scattering in a liquid medium. Using the characteristic signal from a Raman active band, we show that an applied pulsed electric field induces a macroscopic particle reorientation which may be followed by the transient changes induced in the Raman signal. We have chosen a liquid-crystal material as the medium because this allows us to compare the static and dynamic response with other conventional techniques. This comparison indicates the foundation of a new method for characterizing orientational processes, and the dynamics thereof, at the local molecular structure level. The method does not require the use of perturbing dye or spin probes.

Many molecules and particles have anisotropic electric, magnetic and optical properties. Particles in fluid suspension, for example, seem to be optically isotropic because of the randomness of their individual orientation until an external field is applied. Such a field (which may be electric, magnetic, acoustic or optical) induces a partial alignment of the particles and the bulk material becomes optically anisotropic. Thus, because of the field-induced orientation, the bulk properties reflect the anisotropic properties of the individual particles. For such suspensions, electric-field induced changes in the optical properties (that is, scattered intensity¹, birefringence², dichroism², optical activity³ and fluorescence⁴) have led to the establishment of a series of standard techniques of 'electro-optic' characterization.

The use of pulsed fields, particularly, has allowed bulk molecular reorientation to be studied in these systems.

Liquid-crystal systems are, however, more complicated⁵. Structurally they have anisotropic electric, magnetic and optical properties but, because they are highly condensed systems, particle interactions and associations cause local domain structure on the size scale of a few micrometres. Unless suitably aligned, these domains are randomly orientated in space and cause an intense scattering of light giving rise to a milky or turbid appearance. If, however, these materials are contained in a thin cell and surface alignment techniques⁶ are used, they become optically clear and electro-optic phenomena may be observed. Such cells used with low molecular weight organic compounds and suitable optical arrangements form the basis of the liquid-crystal display industry⁷. Because of the condensed nature of the materials, only bulk properties are normally able to be studied and experimental data are referred to a 'director' which is essentially the mean direction or orientation within a domain. An applied electric field will cause director reorientation which will relax back to its original state on removal of the field. Unlike the simple non-interacting particle case, the orientation and relaxation processes now become dependent on the applied voltage, the elastic forces in the medium, as well as the dielectric properties. The situation is complicated because the molecules may not be rigid and, thus, intra-molecular relaxation could occur. Hitherto, the optical dynamics have been limited to relating the director motion to bulk material properties. Using the inherent chemical specificity of the Raman effect which arises from the vibrational modes of the polarizability ellipsoid associated with particular bonds, we can show that transient electro-optic methods may be used to follow the reorientation of the Raman tensor associated with a particular bond in the system.

We have assembled a novel Raman microprobe apparatus (see Fig. 1), based on an argon-ion laser used in the continuous

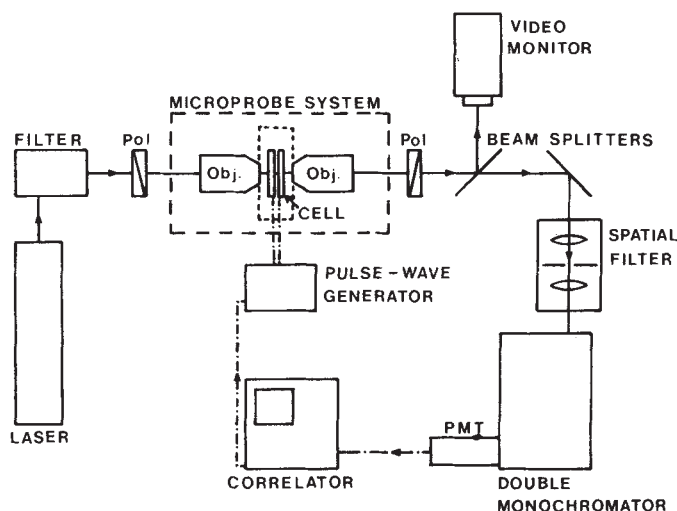


Fig. 1 A schematic diagram showing the Raman experimental arrangement.

wave mode at a wavelength, λ , 488 nm. To avoid spurious heating effects in the samples, the output power was kept below 20 mW. The output beam was then optically filtered to remove laser plasma lines and guided to the cell-microprobe assembly. This assembly used infinity-corrected microscope objectives ($\times 50$ magnification) and was custom built to include glan-laser polarizers (for controlling the polarization plane of the input and scattered light) and spatial filtering (to ensure that only the scattering from the sample volume was detected). The sample holder was an electrically heated constant-temperature oven stable to at least $\pm 0.1^\circ\text{C}$ and capable of holding microscope slides with X - Y translation. The scattered light was then collected in transmission (that is at zero scattering angle) using a further microscope objective ($\times 50$ again) and steered (using prisms and lenses to adjust the input light to the correct numerical aperture) to a Spex 1402 double-grating spectrometer ($f = 7.8$). Light from this spectrometer, which used high-resolution holographic gratings (1,200 grooves per mm), was then detected using a high-gain cooled photon-counting photomultiplier tube. The output signal from the photomultiplier was recorded using a 72-channel digital correlator operating in the signal-averaging mode.

The sample chosen for study was pentyl cyanobiphenyl (5CB), a highly stable liquid crystal exhibiting a nematic phase at room temperature, held in a cell constructed from two conducting glass slides. The cell surfaces were treated with a polyvinyl alcohol film which was then rubbed to give planar alignment⁷. In this way the director was aligned in a controlled manner in the cell and its direction, in the plane of the glass slides, aligned parallel to the input polarization of the argon-ion laser beam. The plane of the transparent glass electrodes was perpendicular to this beam. A voltage applied across the electrodes, therefore, produced a field in the direction of propagation of the beam. When the cell was inserted into the microprobe assembly, an area of $\sim 5\ \mu\text{m}^2$ was illuminated. This was viewed using a video camera and monitor adapted to the collection optics. The video system allowed the condition of the sample to be examined at any time and thus allowed us to verify that any changes in the optical properties recorded were not due to heating or damage to the sample. The sample thickness (and optical path length) was $30\ \mu\text{m}$ and a.c. voltages of between 2 and $6\ \text{V}_{\text{r.m.s.}}$ were applied to the sample. For all the measurements reported here, the voltage was applied either continuously (for the steady-state response) or in 300–800 ms pulses (for the transient measurements).

With the director aligned in the polarization direction of the input light, the Raman active bands studied here gave a maximum response (see Fig. 2a). On application of an a.c.

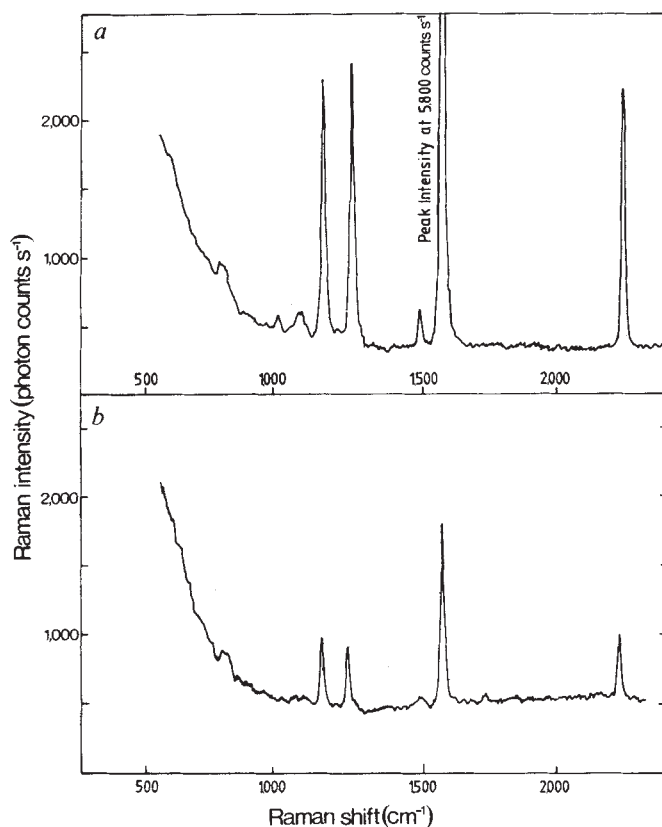


Fig. 2 Raman spectra for 5CB with: *a*, no applied field; *b*, an applied a.c. voltage of $2.2\ \text{V}_{\text{r.m.s.}}$ at 500 Hz. Sample cell thickness was $30\ \mu\text{m}$ and the polarization direction of both the input argon-ion laser beam and analyser for the scattered light was parallel to the director of the nematic phase. Temperature, 25.0°C . Numbers adjacent to the Raman lines correspond to the Raman shift from the exciting line at $\lambda = 488.0\ \text{nm}$. Assignments as follows: $1,179\ \text{cm}^{-1}$, C-H aromatic deformation; $1,285\ \text{cm}^{-1}$, C-C biphenyl link stretch mode, $1,607$, C-C benzene ring stretch mode; $2,224\ \text{cm}^{-1}$, C $\equiv$ N cyano stretch mode. Increasing response at lower wavelengths marks the start of the wing of the Rayleigh scattering.

voltage across the cell, the liquid-crystal molecules orient according to their dipolar structure into the direction of propagation of the laser beam. The Raman tensors, therefore, rotate away from the polarization direction of the beam and the intensity of the Raman signal decreases (Fig. 2b). The strength of the Raman signals, with the steady-state field on, drop to about one-fifth of their field-off values. This reflects the high induced order in the system. On removal of the field, the spectrum relaxes back to its original field-off form. For both the field-on and field-off cases we have verified optically that the samples were not degraded.

Application of pulsed a.c. fields and the high sensitivity of the microprobe technique have allowed us to study time-dependent electro-optic Raman signals in these systems for the first time. A typical transient signal is shown in Fig. 3 for the $1,179\ \text{cm}^{-1}$ line. This response was recorded by monitoring the intensity (photon counts s^{-1}) at a fixed wavelength $517.8\ \text{nm}$ (corresponding to this $1,179\ \text{cm}^{-1}$ line) as a function of time. From Fig. 3 it is clear that the field-on response time ($\sim 110\ \text{ms}$) is faster than the field-free decay ($\sim 400\ \text{ms}$). This is typical of such a liquid-crystal sample. In a normal liquid-crystal cell, the reorientational response time (τ_{on}) is a function of the applied voltage (V). For a planar-planar configuration, as used here, it would be expected, to a good approximation, that $\tau_{\text{on}} \propto V^{-2}$. We have measured τ_{on} as a function of V , Fig. 4, from which it can be seen that τ_{on} is indeed proportional to V^{-2} . Further, the field relaxation time (τ_{off}) should be independent of V . At

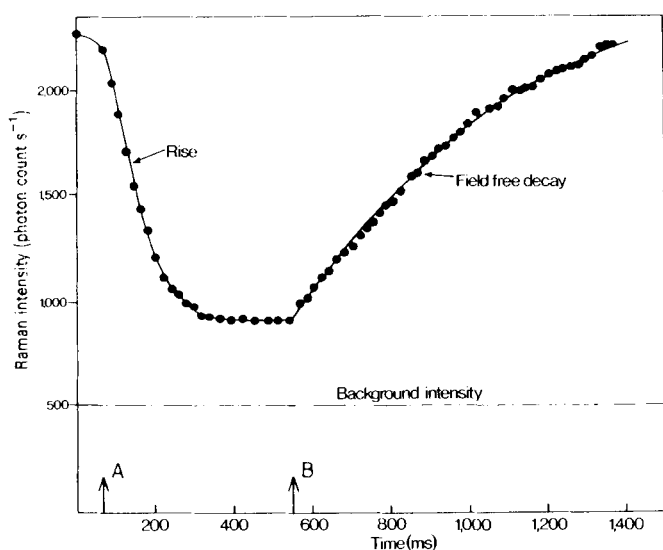


Fig. 3 Transient electro-optic Raman scattering in 5CB. Recorded signal corresponds to the $1,179\text{ cm}^{-1}$ Raman line. A and B denote the start and finish of the AC voltage pulse of amplitude $3.06\text{ V}_{\text{r.m.s.}}$ and frequency 500 Hz . Cell thickness was $30\text{ }\mu\text{m}$; temperature $25.0\text{ }^{\circ}\text{C}$. The polarization conditions were as in Fig. 1.

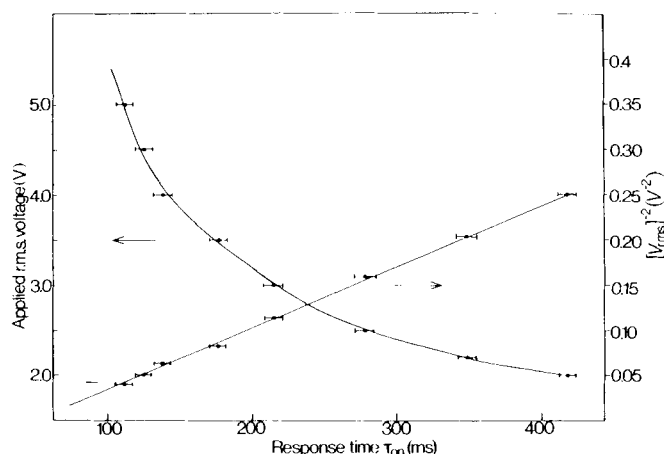


Fig. 4 Transient electro-optic response time (τ_{on}) as a function of the applied root mean square voltage ($V_{\text{r.m.s.}}$) and its inverse squared ($V_{\text{r.m.s.}}^{-2}$). Experimental conditions as in Figs 1 and 2 with the exception of the varied applied voltage. Transient response time is defined as the time for the Raman signal to fall to $1/e$ of the difference between the field-off and field-on saturation values.

constant temperature, τ_{off} was observed to be independent of V . Therefore, we concluded that the transient electro-optic Raman scattering (TEORS) is monitoring orientational motion and relaxation phenomena. The data presented refer to the weaker $1,179\text{ cm}^{-1}$ line which corresponds to a C-H 'deformation' mode in the aromatic ring structure of 5CB. Stronger signals were recorded for the other lines, particularly the $1,607\text{ cm}^{-1}$ C-C 'stretching' mode. However, we have chosen to use the weaker band to illustrate the potential of the TEORS method. All of the bands studied exhibited the same τ_{on} and τ_{off} characteristics. This is expected because these bands are all associated with chemical bonds located in the rigid core structure of the cyanobiphenyl molecule. Although we are now carrying out a detailed analysis of both the τ_{on} and τ_{off} data and applying the method to more flexible molecules, it is evident that the response times reported are typical for such liquid-crystal materials⁷. We have verified this by studying the electro-optic response times

of the unshifted Rayleigh line using crossed polars in the same switching conditions.

We have established a new electro-optic method for characterizing orientational motion in fluid systems using the chemical selectivity of the Raman effect. Because of this selectivity, we do not need to use solvated dye or spin probes to study the orientational processes. If this field-driven technique is combined with the linewidth⁸ and order parameter⁹ methods already established for Raman scattering, then in one apparatus one has all of the experimental features normally only available to methods such as NMR. There are further advantages in that the experiment is much less expensive and allows rapid time dependent phenomena to be studied in real time. The sample volume is small ($\sim\mu\text{m}^3$) and, therefore, any feature that may be observed using a normal optical microscope may be studied in the Raman apparatus. Note that low concentrations of micrometre-sized pollutants have been studied by a time-averaged Raman microprobe system¹⁰. These experiments may now be carried out as a function of time.

We have recently applied our time dependent method to dilute solutions of polymers and polymeric liquid crystals and these results will be reported soon. We also envisage extending the studies to two areas of interest in the field of liquid crystals. The first is in the study of dye-liquid-crystal interactions in the so-called guest-host effect¹¹ where the dye molecules clearly perturb the local molecular dynamics, and the second is in the study of biological liquid crystals. Our original experiment was set up with the intention of studying the motion of proteins in lipid bilayers and cell walls so that the influence of drug additives on the protein dynamics might be monitored. Now that the technique has been established, both of these studies will be carried out and hopefully lead to a better understanding of the importance of local dynamics in such complex condensed systems.

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Dependence of the flexural rigidity of the continental lithosphere on rheology and temperature

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While the first-order mechanical properties of oceanic and continental lithosphere are well defined and explain the observed increase in lithospheric flexural strength with thermal age, the temporal variation of continental flexural rigidity following loading, and its dependence on lithospheric composition and temperature structure, is only just beginning to be appreciated. We describe here the results of a quantitative rheological model in which laboratory-determined rock deformation data and transient

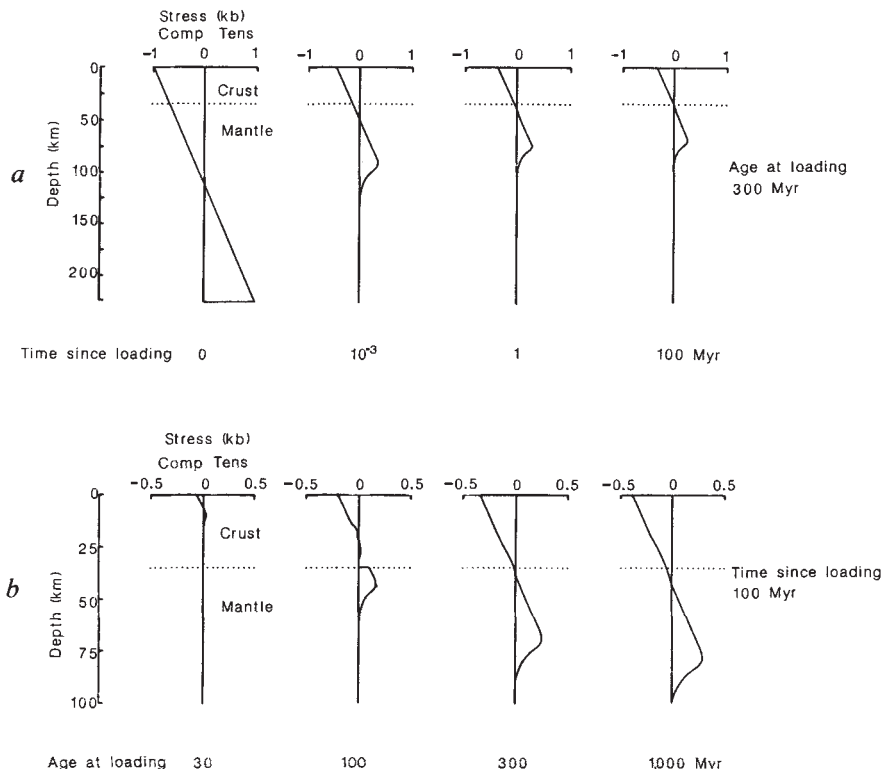


Fig. 1 Horizontal flexural stress plotted against depth: *a*, as a function of time elapsed since loading for lithosphere with a thermal age of 300 Myr; rapid stress relaxation is associated with a corresponding decrease in flexural rigidity; *b*, as a function of thermal age at time 100 Myr since loading. Increasing the thermal age allows the flexural stresses to be supported to a greater depth. In particular, Palaeozoic (300 Myr) and Proterozoic (1,000 Myr) lithospheres are associated with high flexural rigidities for Cenozoic (30 Myr) and Mesozoic (100 Myr) loading respectively.

temperature distributions, resulting from intra-plate basin formation within continental interiors, have been integrated. Continental flexural rigidity is shown to be controlled by crustal thickness, lithospheric thickness through the temperature structure, and the interaction of these factors during basin formation. For thermally young lithosphere, the flexural rigidity is dominated by the quartzofeldspathic rheology of the crust, while for the thermally older lithosphere, it is dominated by the olivine rheology of the mantle. Flexural data, through thermo-mechanical models of lithospheric deformation, provide a powerful constraint on both the transient and steady-state temperature structure of the lithosphere.

Studies of geological loading within the oceans and continents have established that lithospheric flexure is an important mechanism by which emplaced loads are supported¹⁻¹³. The flexural properties of the lithosphere can be parameterized by the flexural rigidity, D , which relates to the effective elastic thickness, T_e by $D = ET_e^3/12(1-\nu^2)$, where E is Young's modulus and ν is Poisson's ratio. Geologically, T_e is the depth to the critical lithospheric isotherm which defines the elastic-plastic transition^{1,5}.

Two fundamentally different quantitative models have been developed to investigate the temporal mechanical properties of the continental lithosphere—the elastic and Maxwell visco-

elastic models—which predict fundamentally different time behaviours of the lithosphere following loading^{6,13}. The elastic model, as modified to reflect the thermal structure of the lithosphere at the time of loading (that is, the thermo-elastic model¹⁰⁻¹²), predicts deflections which are dependent only on the thermal age of lithosphere at the time of loading. In contrast, the Maxwell visco-elastic model is characterized by an initial high flexural rigidity which decreases with time following loading such that only the time since loading is important in defining the flexural response⁶. Flexural stresses created in a Maxwell visco-elastic lithosphere relax asymptotically to zero over geological time. As a consequence of their different time behaviour, the two models predict significantly different basin geometries and stratigraphies^{5,7,9,13}.

While the flexural deformation within the oceanic and continental lithospheres can be successfully analysed using the elastic model, unacceptably high elastic bending stresses² (10–50 kbar), rheological considerations of the way in which rock-forming minerals deform¹⁴, and the failure to predict the observed rapid relaxation from the seismic to an asymptotic elastic thickness¹, invalidates a purely elastic response of the lithosphere to loading. Alternatively, the Maxwell visco-elastic model in which low lithospheric rigidities are predicted 100–

Table 1 Age, load and rigidity parameters of various geological features

Geological feature	Age of load (Myr)	Thermal age of basement (Myr)	Thermal age of basement at time of loading (Myr)	Effective rigidity ($\nu = 0.25$, $E = 10^{12}$ dyn cm ⁻²)	Symbol in fig. 3
Lake Agassiz	0.007–0.014	1,600–1,800	1,600–1,800	5.9×10^{31} – 1.3×10^{32}	1 (ref. 6)
Lake Algonquin	0.00115–0.00125	1,200–1,500	1,200–1,500	2.9×10^{31} – 1.1×10^{32}	2 (ref. 6)
Ganges Basin	2–50	750–1,600	700–1,558	5×10^{31} – 10^{32}	3 (ref. 13)
Appalachian Basin	280–450	900–1,100	450–820	5×10^{31} – 2×10^{32}	4 (ref. 13)
Lake Bonneville	0.015–0.02	24–290	24–290	2.4×10^{29} – 2.9×10^{30}	5 (ref. 6)
Molasse Basin	2–40	270–365	230–363	10^{30} – 10^{31}	6 (ref. 13)
Anadarko Basin	230–330	510–530	180–300	4.1×10^{30} – 1.9×10^{31}	7 (refs 35, 36)
Michigan Basin	280–450	500–800	50–520	4.9×10^{30} – 1.2×10^{31}	8 (ref. 12)
Gulf Coast Basin	2–120	170–190	50–188	1.5×10^{30}	9 (refs 37, 38)
Williston Basin	113–351	500–600	149–487	7.2×10^{30} – 4.0×10^{31}	10 (ref. 31)

200 Myr after loading^{4,6} is equally inapplicable¹². A generalized model of continental lithospheric flexure is needed, therefore, which combines the long-term asymptotic finite rigidity of the thermo-elastic model with the short-term rapid relaxation of the Maxwell visco-elastic model. Such a model, using laboratory-derived creep deformation properties of quartz, feldspars and olivines, is termed a thermo-rheological model and incorporates the temperature and stress dependency of lithospheric material. This model has been used to examine quantitatively the relaxation of flexure-induced stresses with time as a function of depth, geothermal gradient (or lithospheric temperature structure) and crustal thickness within the continental lithosphere. The calculated stress-depth relationships are then used to determine the effective elastic thickness, T_e , and flexural rigidity of the lithosphere as a function of time since loading.

Purely elastic initial horizontal flexural stress is assumed to take the form of $\sigma_x(t=0) = \sigma_0(1 - 2z/L)$ where z is depth, L the initial lithospheric thickness and σ_0 the maximum flexural stress before failure. The model assumes a visco-elastic Maxwell rheology for each infinitesimal component of the lithosphere, that is, for each component, $e_x = \sigma_x/E - \nu\sigma_y/E - \nu\sigma_z/E + e_x^v$ and similarly for e_y and e_z , where e_x is total strain, σ is stress and e_x^v is the viscous creep strain.

Conservation of horizontal force before and after flexure allows the additional constraint,

$$\int_0^L \sigma_x dz = 0$$

By assuming that the infinitesimal layers comprising the lithosphere are welded together during stress relaxation implies that

$$\frac{de_x}{dz} = 0$$

Manipulation of the above equation with the additional constraints of $\sigma_z = 0$ and $e_y = 0$ (plane strain) gives

$$\sigma_x = \int_0^t \left(\frac{1}{L} \int_0^L k \dot{e}_v dz - k \dot{e}_v \right) dt'$$

where $k = E/(1 - \nu^2)$ and

$$\dot{e}_v = (\sigma_x(2 - \nu) - \sigma_y(1 - 2\nu))/6\eta$$

where η is the apparent viscosity. The derivation is described in greater detail in ref. 15.

Temperature and power-law stress-dependent ductile deformation is assumed to be controlled by dislocation and Dorn law creep of olivine in the mantle¹⁶⁻¹⁸, while crustal creep is modelled as the dislocation creep of dry (lower crust) and wet (upper crust) quartz¹⁹. Dislocation creep takes the mathematical form $\dot{e}_v = A \exp(-E/RT) \tau^n$ where τ is shear stress, T is temperature (K) and A , E and n are material constants. The upper crust is assumed to contain 50% quartz and the lower crust only 10% quartz.

Stress modification by near-surface brittle failure of the crust has not been included in the model so that the primary thermo-rheological effects controlling flexure can be clearly demonstrated. Upper lithospheric brittle failure, when included in the model, results in only a minor reduction in flexural rigidity. Rigidity, therefore, as calculated here, represents an upper bound.

The lithospheric geothermal gradient has a critical role in controlling the strength of the lithosphere because of the temperature-dependent rheology of the crust and mantle. To span the range of acceptable geothermal models, geotherms have been calculated using cooling plate models²⁰⁻²⁵ with plate thicknesses of 125 and 225 km. Radiogenic heat productivity distributions given by Slater *et al.*²³ and Nicolaysen *et al.*²⁴ have been included in the 125- and 225-km models respectively. An initial uniform plate temperature of 1,333 °C is assumed²⁵. With time, the lithosphere cools progressively by conduction so that the lithospheric temperature structure is directly related to its thermal age^{12,26}.

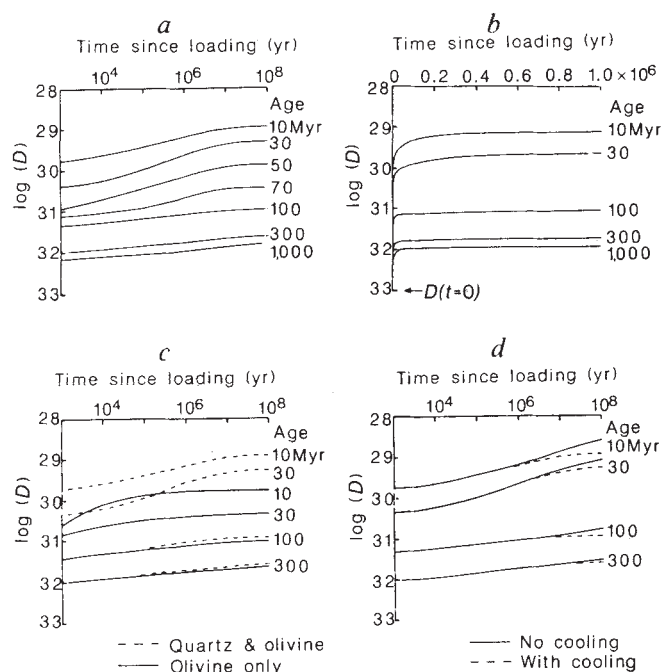
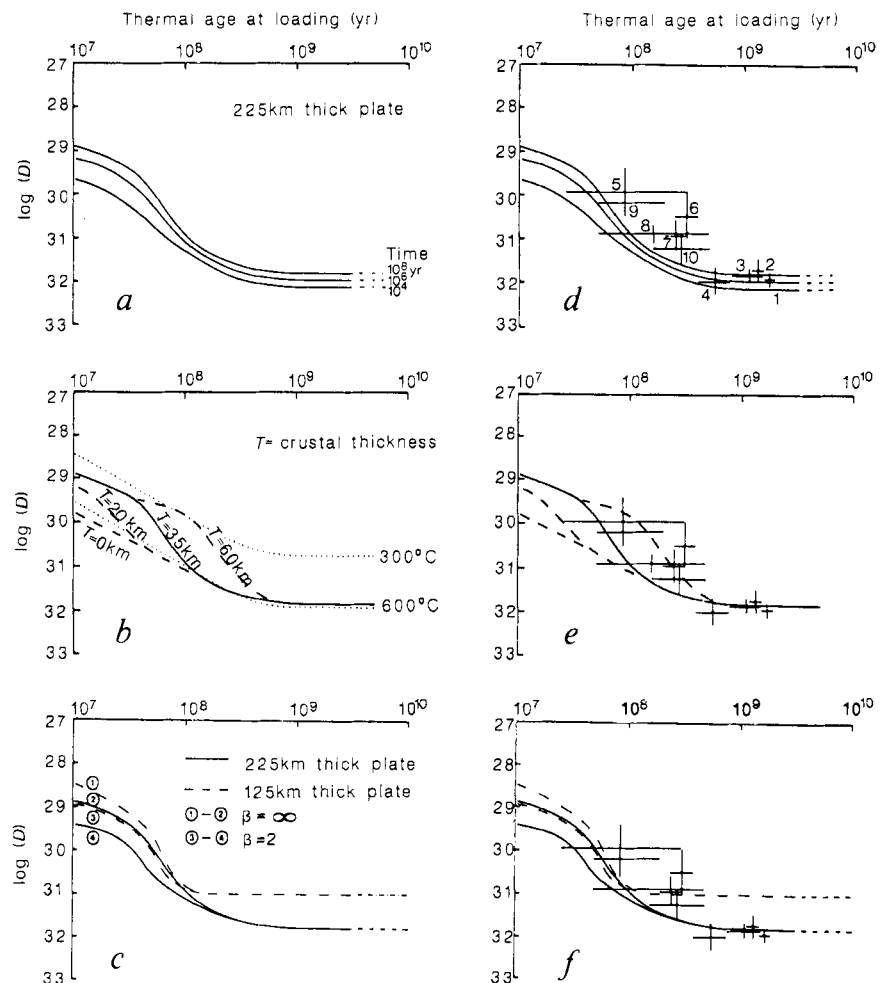


Fig. 2 The flexural rigidity, D : *a*, plotted against time since loading for lithosphere of various thermal ages with quartz rheology crust and olivine rheology mantle; the rate of relaxation towards the asymptotic rigidity increases with decreasing thermal age of the lithosphere; *b*, plotted against linear time; the rapid relaxation period, regardless of thermal age, is < 0.2 Myr; *c*, compared with an all-olivine rheology lithosphere; the increase in the olivine component accelerates the flexural rigidity stabilization; *d*, compared with a model in which the geothermal gradient has remained constant since loading. This (secondary) effect is to reduce the value of the asymptotic rigidity. Lithospheric cooling therefore, leads to a more rapid stabilization of flexural rigidity by 'locking in' flexural stresses.

Figure 1*a* shows horizontal flexural stresses at various times after loading for lithosphere with a thermal age of 300 Myr (equivalent to Hercynian-aged lithosphere) and crustal thickness of 35 km. The initial flexural stress has a maximum compressive value of -1 kbar at the surface ($\sigma_0 = -1$ kbar). With time, ductile deformation results in stress relaxation within the lower lithosphere, reducing the depth extent over which the stresses are supported. Figure 1*b* shows the horizontal stress remaining after 100 Myr since loading for a range of lithospheric thermal ages. The older, hence colder, lithosphere shows stress supported to increasingly greater depths, corresponding to greater effective elastic thicknesses. Rheological variations associated with the Moho and at mid-crustal levels can be seen to generate discontinuities within the stress-depth relationships. The model described above conserves plate curvature during stress relaxation. An alternative condition in modelling flexure, more applicable to the lithospheric response to superimposed loads, would be the conservation of bending moment. For such a condition, stress relaxation in the lower lithosphere is accompanied by stress amplification in the upper lithosphere in order for the lithosphere to support the load. While the two models predict different stress levels, the resultant flexural rigidities are expected to be similar.

Rather than tracking lithospheric stress, it is advantageous to measure the variation in lithospheric flexural rigidity as it is this parameter which characterizes the surface deformation of the lithosphere. A simple estimate of flexural rigidity is obtained from the depth to the neutral fibre. In Fig. 2*a*, flexural rigidity is plotted against the logarithm of time since loading for lithospheric models with various thermal ages. An inverse relationship exists between flexural rigidity and the time since loading, whereas it increases with increasing lithospheric thermal age.

Fig. 3 Modelled flexural rigidity predicted by the 225-km plate thermo-rheological model as a function of the thermal age of the lithosphere at the time of loading: *a*, for elapsed times 10^4 , 10^6 and 10^8 yr after loading; *b*, showing the effects of crustal thickness variation; the predictions of the thermo-rheological model are compared with those of the thermo-elastic model for the 300 °C and 600 °C isotherms; *c*, compared with the predictions of the 125-km plate model; curves for stretching by $\beta = 2$ and $\beta = \infty$ (the oceanic plate cooling model) are shown; *d-f*, as for *a-c* but with superimposed observations of continental flexural rigidities as a function of the lithospheric thermal age at the time of loading.



For thermally young lithosphere, flexural rigidity rapidly stabilizes (within 10^6 yr) after loading, while thermally older and colder lithosphere takes longer. Lithosphere of 1,000 Myr thermal age exhibits a slow but continuous decrease in flexural rigidity with time. The plots of Fig. 2*a* using a logarithmic time axis are, however, misleading with respect to the rate of the initial relaxation phase. Figure 2*b* shows the time since loading plotted linearly against flexural rigidity and indicates that the rapid relaxation of flexural rigidity actually occurs within the first 0.2 Myr following loading, after which it slowly approaches its asymptotic value.

Comparison of the post-loading behaviour of the lithospheric rheological models with both composite quartz-olivine (crust-mantle) and all olivine compositions (Fig. 2*c*) indicates that an olivine lithosphere approaches its asymptotic flexural rigidity significantly more rapidly than a quartz-olivine lithosphere. This suggests that oceanic flexural rigidity should stabilize more rapidly than that of the continental lithosphere and, therefore, that it is flexurally stronger than continental lithosphere of the same thermal age.

A contribution to the stabilization of flexural rigidity arises from the continued cooling of the lithosphere following loading. Its significance is summarized in Fig. 2*d* which shows that the rate of rigidity stabilization is increased for oceanic lithosphere and thermally young continental lithosphere, both of which cool rapidly (it is this rapid cooling which essentially 'freezes in' the effective elastic thickness²⁷). However, this freezing is, in effect, of secondary importance relative to the main controlling factor on flexural rigidity stabilization, which is the stress decay within a material characterized by a temperature- and stress-dependent power-law rheology.

The flexural rigidity of the lithosphere tends to increase with its thermal age (Fig. 3*a*), a result which is consistent with flexural results from both the oceans and continents^{1,12}. Fig. 3*a* shows

separate curves for 10^4 , 10^6 and 10^8 yr after loading. The kink in the curves at 100 Myr results from the dual quartz-olivine rheology of the continental lithosphere. The contributions of the quartz and olivine rheologies are illustrated in Fig. 3*b*, where flexural rigidity is plotted against the thermal age of the lithosphere at the time of loading for models which consist of composite quartz-olivine (crust-mantle) and all olivine compositions. For thermally younger, hotter lithosphere, the flexural rigidity is controlled by the crustal quartz rheology while for older, cooler lithosphere (>200 Myr), the flexural rigidity is controlled by the olivine rheology. Flexural rigidity increases rapidly in the region of 100 Myr thermal age as the quartz rheological control gives way to that of olivine. As quartz has such an important role in lithospheric flexure^{12,28}, so must, therefore, the crustal thickness. Figure 3*b* shows the effect of changing crustal thickness ($T = 0, 20, 35$ and 60 km) and, not surprisingly, increasing the crustal thickness tends to decrease the flexural rigidity of the continental lithosphere until control is again passed back to the mantle olivine component for large thermal ages. In Fig. 3*b*, the predictions of the rheological model are also compared with those of a simple thermo-elastic flexure model¹³ in which the effective elastic thickness is controlled by a critical isotherm defining the elastic-plastic transition. For thermally younger lithosphere, when quartz rheology controls lithospheric strength, the 350 °C isotherm is the critical isotherm while for thermally older lithosphere, when olivine rheology is more important, it is the 550 °C isotherm.

Many intracratonic basins of continental interiors are characterized by finite stretching of the lithosphere²⁹⁻³⁶ and, as such, will modify the temperature and hence mechanical structure of the lithosphere. Stretching in our context increases the surface heat flow by a factor β during the rifting process²⁹. In fig. 3*c*, the pre-rift lithosphere has a continental average heat flow²² of 60 mW m^{-2} . The resulting thermal perturbation is important in

defining the transient temperature structure of the continents. The variable spatial and temporal distribution of intra-continental rifting may, however, result in a complex temperature structure²², but with each rifting event, the thermal perturbation will decay by conductive cooling of the continental lithosphere regardless of previous history or spatial complexity. Figure 3c shows the relationship between the stretching factor responsible for resetting the continental lithospheric thermal structure and the corresponding (post-stretching/rifting) temporal variation in flexural rigidity for values appropriate to basin formation ($2 < \beta < \infty$). The effect of increasing β is to reduce the flexural rigidity early in the post-stretch period, the effect rapidly diminishing with time.

The temperature structure of the lithosphere is strongly dependent on its assumed thickness and radioactive distribution. To contrast the 225-km lithospheric thickness model (with the radioactive distribution of Nicolayson *et al.*²⁴—representing an upper bound) so far used, a 125-km lithospheric thickness model (with the radioactive distribution of Sclater *et al.*²³) has also been considered (Fig. 3c). The 125-km plate model predicts significantly lower flexural rigidities relative to the 225-km plate model for thermal ages $> 10^8$ yr.

The temporal variation in flexural rigidity of the continental lithosphere is strongly modified by crustal thickness (quartzic component), lithospheric thickness (olivine component and thermal time constant) and the interaction of these factors during basin formation (lithospheric stretching and transient temperature structure). Observations of continental flexural rigidity (for which both the age of the load and thermal age of the basement are known; Table 1), when plotted against the thermal age of their respective basements, are not compatible with any one thermal/lithospheric model (Fig. 3d–f). Realistic variations in crustal thickness and lithospheric stretching lead to modelled rigidities which at least help us to understand the scatter within the observations.

The loading of thermally old lithosphere ($> 10^8$ yr) is associated with essentially a steady-state lithospheric temperature structure. However, loading of thermally young lithosphere (the lithospheric temperature structure, therefore, being transient) results in considerable scatter in the observed rigidities which, in part, is related to either crustal thickness or lithospheric stretching variations (Fig. 3d, e). The remaining scatter is almost certainly a result of the inability to assess accurately the past or present thermal age of the continental lithosphere, either because of the failure to reset radiometric ages within the upper crust (or that the reset region has yet to be exposed by erosion) or possibly because radiometric age resetting lags significantly behind load reactivation (that is rigidity resetting) due to the thermal diffusivity of the lithosphere. For example, even though the Molasse Basin developed on Hercynian crust, as evidenced by recovered basement in Austrian and Swiss exploration wells, the apparently anomalous flexural rigidity for the basin (see Fig. 3a, Table 1) may reflect a bias introduced by thermal activity associated with either the formation of the Rhine Graben and/or major Tertiary–Quaternary volcanic activity within Europe (for example, Chaîne des Puys, Kaiserstuhl, Vogelsberg and northern Bohemia).

If the temperature structure of the continental lithosphere is governed by a cooling plate model, a unique steady-state temperature structure for old lithosphere, and thus a unique asymptotic flexural rigidity, is a reasonable expectation. While variations in crustal radioactive heat production may produce significant heat flow anomalies, they have a negligible effect on sub-crustal lithospheric temperature and hence flexural rigidity. The grouping of reliable flexural rigidities for thermally old continental lithosphere is consistent with a plate model for the thermal evolution of the lithosphere and further, suggests a steady-state lithospheric thickness of 175–225 km.

Although our discussion began with flexural rigidity, it concludes with a statement on lithospheric temperature structure. Flexural data, therefore, through thermo-mechanical modelling of lithospheric deformation, provides a powerful constraint on

both the transient and steady-state temperature structure of the continental lithosphere. The thermo-elastic model^{1,2,12} satisfies the general thermal age dependency as indicated by flexural observations, but the detailed time evolution of flexural rigidity after loading, which controls the details of sedimentary basin geometry and stratigraphy, can only be addressed by a thermo-mechanical flexural model which integrates laboratory-determined rock deformation data and transient lithospheric temperature structures associated with either intracratonic sedimentary basin formation or continental collisions, such as presented here.

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Fossilized polyps in 430-Myr-old *Favosites* corals

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Tabulate corals are among the more characteristic fossils of the Silurian period, but because only their skeletons have been preserved, their taxonomic position and mode of life have been uncertain. Although they are generally placed¹ in the phylum Coelenterata (Cnidaria), the recent discovery of living sclerospores that share architectural affinity with some tabulates, has renewed debate about the evolutionary relationships of the latter. In particular, the tabulates have been assigned^{2,3} to the sponges (phylum Porifera). I report here the discovery of fossilized polyps in a *Favosites* from the Silurian of Quebec. This incontrovertibly demonstrates that the tabulates belong to the phylum Cnidaria, and details of the soft-part morphology suggest that the Tabulata form a group distinct from other corals.

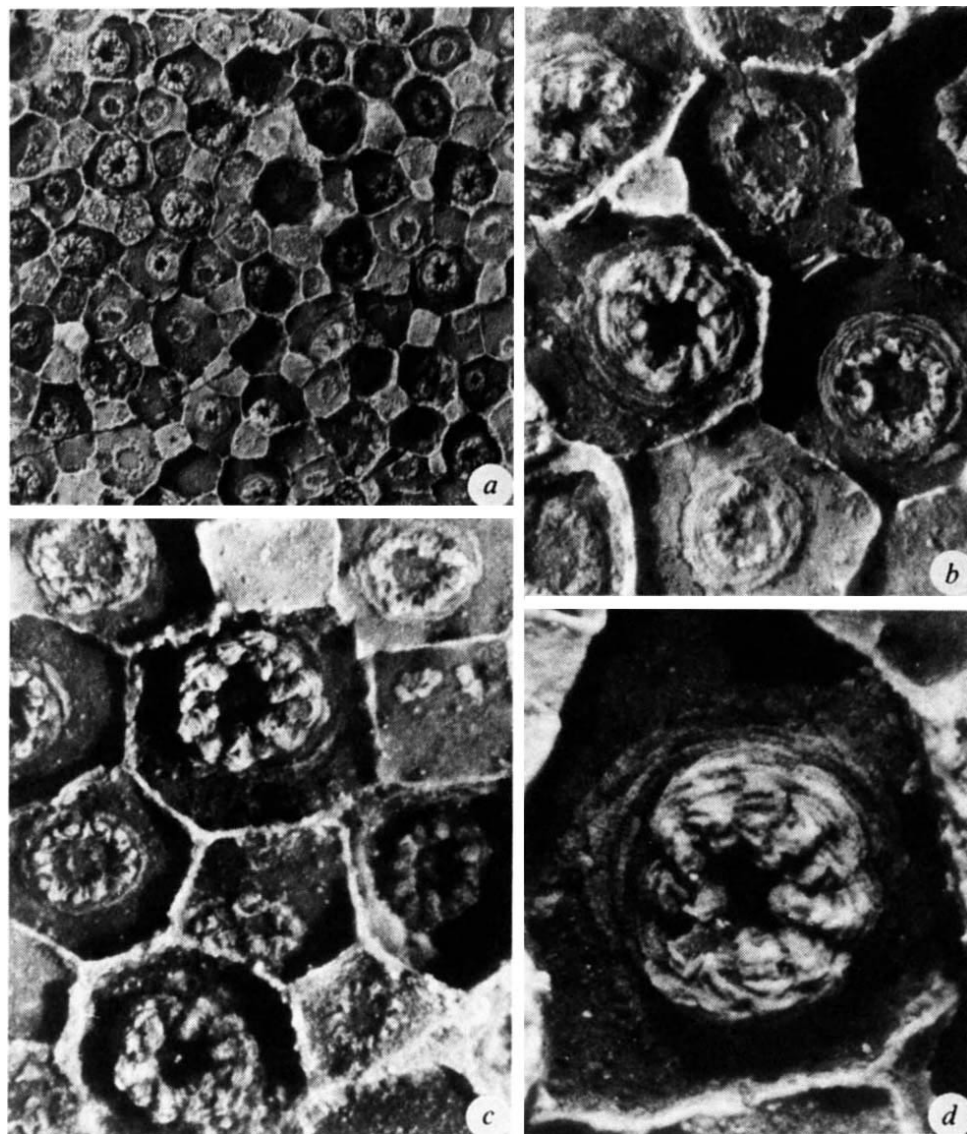


Fig. 1 Calcified polyp structures preserved in the calices of a *Favosites* corallum from the Jupiter Formation (Silurian, Llandoveryan) of Anticosti Island, eastern Canada (specimen GS79441, Geological Survey of Canada, Ottawa). *a*, Overview of the hemispherical colony showing 109 corallites, most with centred polyps ($\times 3.75$); *b*, *c*, detailed view showing varied stages of polyp degradation ($\times 12.75$); *d*, inwardly retracted tentacles and gut cavity and concentrically layered, radially striated view of the lower polyp stalk ($\times 33.75$).

Favosites, like most of the tabulate corals, secreted a skeleton of calcite, unlike the modern scleractinians which favour aragonite. Some modern anthozoans, such as sea anemones, produce sizable, 60-mm diameter, trochoid chitinous shells resembling gastropod conchs⁴. However, there seems to be no record of calcified, silicified or phosphatized polyps. Polyp tissues are soft and contain no apparent mechanism for *in situ* precipitation of calcium carbonate. This is produced only by the basal disk tissues that secreted the calicular wall, septa and tabulae. The discovery of unmistakable calcified polyps, therefore, is both surprising and exciting.

Six well-preserved coralla of previously undescribed species of *Favosites* were collected from the basal 50 m of the Jupiter Formation (early Silurian, Llandoveryan, C¹-C²) of Anticosti Island, Quebec, Canada. Materials came from two different localities: A55, NTS 12° E/11° W 74800:89170 and A98, 12° E/12° E 53760-820:34770-880 in the central and southern parts of Anticosti. The specimen illustrated in Fig. 1 comes from the second locality (A98), in the basal 10 m of the Jupiter, a coastal bluff section of richly fossiliferous calcareous shales with interbedded, thinly bedded, micritic limestones. The fauna includes the brachiopods *Eocoelia hemisphaerica sefinensis*, *Atrypa* (*Gotatrypa*), *Triplesia anticostiensis*, *Pentamerus oblongus*, *Leptaena*, and small matchstick bryozoans along with small, hemispherical *Favosites*. No other corals, stromatoporoids or calcareous algae were found. The fauna is consistent with a late Llandovery (C¹-C²) age. The graptolite *Monograptus sedgwicki*

has been found some 40 m higher in the section. The type locality for the boundary between the Jupiter Formation and the underlying Gun River Formation is adjacent to the discovery site.

The corallum showing the best polyp preservation is hemispherical and 6 cm in diameter. Corallite diameters vary from 1.5 to 2.5 mm, with mural pores in the centre of the walls, variably developed short septa and very thin corallite walls (as verified in thin sections). The calices were freed from enveloping sediment by immersing the corallum in Quaternary O for 1 h and cleaning in an ultrasonic bath for 80 min.

More than 200 of the corallites on the surface of the specimen in Fig. 1 showed the attachment of polyp structures preserved as calcite. Polyps were seen in a 'retracted' position, that is with the tentacular ring inwardly bent and tentacles inwardly directed. Tentacles tapered and were blunt-ended. Of more than 300 corallites measured in one corallum, ~70% had polyps preserved in varied stages of degradation from only the remnants of a basal disk, to those with shrunken tentacles, to fully displayed structures (Fig. 1). The fossilized polyps occupy about two-thirds of the calice: this is not significant because the fully extended polyps of living corals can be inflated to several times the corallite diameter. The concentric wrinkles at the base of the polyp stalk and fine radial elements similar to those seen in modern corals⁵ were also preserved (Fig. 1d).

The average number of individual tentacles measured for well-preserved polyps was 12; a few had 11 or 13 tentacles. No particular enlargement, division or symmetry of tentacles was

observed, suggesting that the tentacles were uniformly sized in *Favosites*, that is no 'sweeper' tentacles were indicated. The number of septal spines in the thin sections of *Favosites* examined was difficult to study as septal insertion was uneven or arrhythmic, and the septal spines were upwardly directed. However, *Astrocerium*, a close Silurian relative of *Favosites*, is characterized by 12 regular rows of septa. The Silurian heliolitid tabulates are also typified by 12 septa. The significance of the relationship between the discovery of 12-fold tentacles and the occurrence of 12 septa in several groups of tabulates is unclear. There may be no direct relationship.

The discovery of tentacular polyps in *Favosites* discards the possibility that the Favositidae, and probably other tabulates are aberrant members of the Phylum Porifera, as has been suggested recently. Their taxonomic affinities seem to lie clearly with the Cnidaria.

The development of a common scheme of 12 tentacles for each polyp suggests the possibility of dodecal symmetry in the Favositidae. This, along with the known presence of 12 septa in other tabulates, seems to set the Tabulata away from the tetracorals (Rugosa), hexacorals (Scleractinia) and octocorals (Octocorallia).

The method of polyp calcification is as yet undetermined. In the absence of unusual environmental conditions on the stable marine carbonate platform of Anticost Island during the Silurian, it is tempting to suggest that algal symbionts were present in the tabulates and that these may have initiated precipitation of calcite nuclei or spicules inside the polyp. There is no evidence for this at present, as it is not clear whether calcification occurred while the polyp was alive or immediately after death. If algal symbionts were pervasive in the tabulates it is difficult to understand why polyp preservation is so rare.

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Paternal cyclophosphamide treatment of rats causes fetal loss and malformations without affecting male fertility

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The use of cytotoxic, mutagenic and carcinogenic agents as treatment for various types of cancer may be particularly hazardous in men of reproductive age as there exists the possibility that this may lead to congenital malformations in the progeny. Such agents can affect fertility and other aspects of male reproductive function, for example, treatment with anti-cancer drugs such as cyclophosphamide has been associated with oligozoospermia, azoospermia and increased levels of serum follicle-stimulating hormone (FSH)^{1,2}. Depending on the cumulative dose and the duration of treatment, spermatogenesis often returns but this may take years^{3,4}. The relevance of the effects of such chemicals on the male reproductive system to the offspring is poorly understood. We have set out to determine whether present tests of male reproductive function (that is, endocrine status, numbers of spermatozoa, fertility) can predict deleterious effects of a paternally administered agent on the offspring. Here, we report that chronic administration in rats

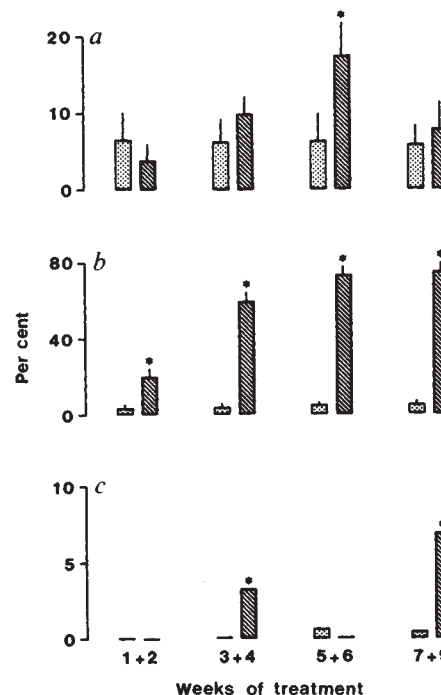


Fig. 1 Effects on the progeny from matings at specified times after initiation of paternal treatment with saline (□) or 5.1 mg per kg per day of cyclophosphamide (▨) by gavage. The mating schedule used allows an assessment of the stage of spermatogenesis affected by cyclophosphamide. Effects on the progeny after 1-2 weeks of treatment of the male indicate that epididymal spermatozoa were affected; similarly, after 3-4 weeks the germ cells affected were spermatids; after 5-6 weeks spermatocytes; and after 7-9 weeks spermatogonia⁷. Pre-implantation loss (a) is defined as the number of corpora lutea minus the number of implantations divided by the number of implantations. Post-implantation loss (b) is defined as the number of implantations minus the number of resorptions divided by the number of implantations. Both these values are expressed as per cent mean per litter. Fetuses were examined for gross malformations. External malformations (c) are expressed as the per cent of malformed fetuses divided by the total number of live fetuses for a given time period. Control and treatment groups were compared using a Fisher exact test¹⁰. The level of significance is taken as $P \leq 0.05$ throughout.

of low doses of the widely used drug cyclophosphamide had minimal effects on the male reproductive system and fertility, but resulted in malformations and retardation of growth in the surviving fetuses and a high frequency of fetal death. Thus, adverse effects on the fetus cannot be predicted from the effects of a drug on the male reproductive system.

Cyclophosphamide (CP) was chosen for this study as it is widely used clinically as an anti-cancer agent and has also been associated with variable periods of infertility in humans³. Adult male Sprague-Dawley rats were treated daily six times a week for 11 weeks by gavage with either saline solution (control) or one of three doses of CP (1.4, 3.4 and 5.1 mg per kg per day). These daily low-dose regimens simulated maintenance regimens used clinically for cancer treatment⁵. The 77-day treatment period covers one full cycle of the seminiferous epithelium (51.6 days) as well as the time needed for epididymal transit of the sperm (~10 days)^{6,7}. Thus, effects that are seen after the first 1-2 weeks of treatment reflect an action of the drug on spermatozoa undergoing maturation in the epididymis. Effects first seen 3-4 weeks after the initiation of treatment reflect action of the drug on spermiogenesis (spermatid development) or post-spermiation changes, while effects first seen after 5-6 weeks of treatment indicate an effect on spermatocytogenesis or subsequent stages. An effect that selectively occurs 7-9 weeks after initiation of treatment would suggest strongly an effect on the proliferative spermatogonial phase of spermatogenesis.

The male rats were killed after 11 weeks and the effects of

Table 1 Effects of chronic treatment with cyclophosphamide for 11 weeks on the reproductive system of male rats

Treatment group	Body weight (g)		Tissue weight (g)			Serum hormones (ng ml ⁻¹)		
	Initial	Final	Ventral prostate	Cauda epididymis	Sperm reserves ($\times 10^6$)*	LH	FSH	Testosterone
Saline	317 $\pm$ 4	500 $\pm$ 14	0.57 $\pm$ 0.08	0.57 $\pm$ 0.02	152 $\pm$ 15	0.52 $\pm$ 0.06	193 $\pm$ 28	2.6 $\pm$ 0.6
Cyclophosphamide								
1.4 mg per kg per day	319 $\pm$ 5	534 $\pm$ 11	0.61 $\pm$ 0.06	0.52 $\pm$ 0.03	188 $\pm$ 17	0.43 $\pm$ 0.09	186 $\pm$ 28	2.2 $\pm$ 1.0
3.4 mg per kg per day	317 $\pm$ 3	481 $\pm$ 24	0.59 $\pm$ 0.05	0.59 $\pm$ 0.02	166 $\pm$ 15	0.36 $\pm$ 0.06	174 $\pm$ 21	1.8 $\pm$ 0.3
5.1 mg per kg per day	325 $\pm$ 4	484 $\pm$ 16	0.48 $\pm$ 0.04	0.59 $\pm$ 0.03	177 $\pm$ 17	0.38 $\pm$ 0.07	198 $\pm$ 13	2.4 $\pm$ 0.8

The serum concentrations of luteinizing hormone (LH), FSH¹⁸ and testosterone¹⁹ were measured by radioimmunoassay. For LH, NIH RP-2 preparations were used as standards. Numbers of spermatozoa in the cauda of the epididymis were determined by the method of Robb *et al.*²⁰ as modified by Scheer and Robaire¹⁹. Data were analysed by one-way analysis of variance with the new Duncan's multiple-range test²¹. Values are the mean $\pm$ s.e.m. The level of significance is taken as $P \leq 0.05$ throughout.

* Sperm reserves represent spermatozoal numbers in the cauda of the epididymis.

the drug treatment were assessed on the basis of body and reproductive organ weights, serum hormone levels and epididymal spermatozoal numbers. To assess the effects of the treatment on the progeny, each male ($n = 6$ per group) was mated overnight with two pro-oestrous females on day 7 of weeks 1 to 7 and 9 of treatment. Males were not treated with CP on the day of mating in order to preclude a direct effect of the drug on fertilization and post-fertilization events by transfer via the seminal fluid⁸. Females ($n = 384$) were killed 20 days after mating and caesarian sections were performed. The rate of embryo or fetal loss was determined by counting numbers of corpora lutea, implantations and resorptions, and the live fetuses were examined for external malformations.

The effects of the drug treatment on the male reproductive system of the rat are shown in Table 1. The similarities of initial and final body weights between the control and treated groups show that the treatment did not apparently affect the general health of the animals. There was no significant change in the weights of the male reproductive organs, in reserves of spermatozoa or in serum hormones reflecting male endocrine status in any of the CP-treated animals (Table 1). Ventral prostate and caudal epididymal weights were unchanged; testis, seminal vesicle and pituitary weights were also similar in the different groups (data not shown). As CP is an alkylating agent that causes most damage to rapidly dividing cells, the drug was expected to affect the seminiferous epithelium and reduce the number of spermatozoa produced. The number of spermatozoa in the cauda of the epididymis provides a good estimate of spermatozoal reserves⁹; clearly these reserves were not affected by the treatment regimen. These results suggest that the proliferative phase of spermatogenesis is unaffected by the cytotoxic effects of CP.

The mating ability and fertility of the males was similar in all groups, as assessed by counting seminal plugs on the morning after mating and the number of pregnant females per group. The number of plugs was similar for all treatment groups (in the range 3.6 ± 0.2 to 4.0 ± 0.2 ; mean $\pm$ s.e.m.). The number of pregnant females per sperm-positive females also did not differ between groups (91–97%). Clearly, the daily doses of CP used in these experiments did not affect the weight gain, endocrine status, spermatozoal reserves or the fertility of the treated males.

In view of the minimal effects of the CP treatment on the male reproductive system, the varied and complex effects on the progeny were quite unexpected. For clarity, only the results for the control and the group treated with 5.1 mg per kg CP are illustrated (Fig. 1). Results for the groups treated with 1.4 and 3.4 mg CP per kg fell between those for the control group and the groups treated with 5.1 mg CP per kg, in a dose-dependent fashion for all parameters. The control level of pre-implantation loss was relatively constant over the 9 weeks of mating (mean 7%, range 3–11%). Pre-implantation loss was low during the first 2 weeks of CP treatment, gradually increased between weeks 3 and 6, and was significantly ($P \leq 0.01$) greater than the control by weeks 5–6. However, this increase disappeared by weeks 7–9.

Thus pre-implantation loss occurred when germ cells were first exposed as spermatocytes or late spermatids.

There was a dramatic dose-dependent increase in post-implantation loss after week 2 ($P \leq 0.001$) for all animals receiving CP (Fig. 1); after week 4 a plateau of 80% loss was reached and maintained until weeks 7–9 ($P \leq 0.001$). The most striking increase in post-implantation loss occurred between weeks 2 and 4 when germ cells were first exposed as spermatids. To determine the timing of post-implantation loss, resorptions were weighed and defined as early (< 100 mg) or late (> 100 mg). Analysis of the weights of the resorptions revealed that this increase in post-implantation loss was due mainly to an increase in early resorptions; 87% of resorptions occurred early in gestation in the control group whereas 98% occurred early in the CP-treated group. Thus paternal treatment with CP resulted in germ-cell damage that was incompatible with development beyond organogenesis in a high percentage of embryos.

The CP-induced pre- and post-implantation losses resulted in a dose- and time-dependent decrease in the number of live fetuses per litter. While no increase of abnormalities was apparent in live offspring from matings at 1–2 weeks (control = 0/196, CP-treated = 0/184) and 5–6 weeks (control = 1/151, CP-treated = 0/55), the frequency of abnormalities was increased in offspring from matings at 3–4 weeks (control = 0/231, CP-treated = 3/93; $P < 0.02$) and at 7–9 weeks (control = 1/254, CP-treated = 4/57; $P \leq 0.005$). The most common abnormalities seen were hydrocephalus, micrognathia and oedema. There was also an increase in the percentage of growth-retarded or low-weight fetuses (fetuses weighing $< 75\%$ of the mean weight for their group) in the offspring from matings at 3–4 weeks (control = 1.3%, CP-treated = 4.3%, $P \leq 0.10$) and at 7–9 weeks (control = 1.2%, CP-treated = 7.0%; $P \leq 0.02$, Fisher exact test¹⁰). (The dose of CP in all the above treatments was 5.1 mg per kg.)

Thus, paternal treatment with CP causes pre-implantation loss when germ cells were first exposed as spermatocytes or late spermatids. Post-implantation loss increased at the greatest rate when germ cells were first exposed as spermatids. There was a significant increase in external malformations and growth-retarded fetuses among the offspring when germ cells were first exposed as either spermatids or spermatogonia. Clearly, the low numbers of malformed fetuses involved make it difficult to elucidate the stage(s) of spermatogenesis affected. Different underlying mechanisms, both genetic and non-genetic, could be postulated to explain the effects, based on the fact that germ cells are first exposed to CP when they are undergoing different processes: spermatozoal maturation (1–2 weeks), cytoplasmic reorganization and chromatin condensation of spermiogenesis (3–4 weeks), meiosis during spermatocytogenesis (5–6 weeks) and active mitotic division (7–9 weeks).

Obviously, there are some severe qualitative changes in the spermatozoa of the treated males that are not manifested by concomitant quantitative changes. The effects seen are probably not due to a cumulative effect of CP in seminal fluid as: (1) the

drug has a short half-life in rodents and man and is eliminated within hours of ending chronic treatment^{11,12} (our protocol allowed 36 h between drug administration and mating); and (2) in the present study, there was no cumulative increase in pre-implantation loss and external malformations with increasing time of paternal drug exposure.

Previous studies in rats indicate that, after paternal treatment, CP can cause a decrease in litter size^{13,14} and lead to dominant lethal mutations¹⁵ as well as behavioural abnormalities in the surviving offspring¹⁴. Although the mechanism(s) underlying paternally mediated effects of CP on the outcome of pregnancy remains unresolved, one possibility is that this drug affects the genetic composition of the spermatozoa—CP has been shown to induce sister chromatid exchange and chromosomal aberrations in male germ cells^{16,17}. In addition, clinical studies have indicated that CP can affect the human male reproductive system¹. As far as we are aware, no study to date has attempted to relate effects on the male reproductive system to the outcome in the progeny. Thus, we have demonstrated, for the first time, that paternal treatment of rats with a commonly used anti-tumour drug can cause malformations and growth retardation of the surviving offspring and lead to dramatic embryo loss, while at the same time having minimal effects on the male's endocrine status or spermatozoal reserves.

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The J1 glycoprotein—a novel nervous system cell adhesion molecule of the L2/HNK-1 family

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The neural cell adhesion molecules L1 (refs 1, 2) and N-CAM (refs 3–5) share a common carbohydrate epitope that is recognized by the monoclonal antibodies L2 and HNK-1 (ref. 6). The L2/HNK-1 epitope is also present on the myelin-associated glycoprotein (MAG) which is thought to mediate surface interactions between the axon and myelinating cell⁷. Other, as yet unidentified,

cell-surface glycoproteins are recognized by the two antibodies and are believed to belong to a family of neural cell adhesion molecules^{1,2,6}. To test this hypothesis, we have prepared polyclonal antibodies to a prominent member of the L2/HNK-1 family, the 160K (relative molecular mass (M_r)160,000) glycoprotein. Here we report that these antibodies, designated J1 antibodies, react with astrocytes and oligodendrocytes and interfere with neurone-astrocyte adhesion, but not with neurone-neurone or astrocyte-astrocyte adhesion. This result suggests the involvement of the J1 antigen in cell-cell interactions.

J1 antibodies do not react with L1, N-CAM or MAG (Fig. 1a–c), but react specifically with the 160K component of 'rest L2' (ref. 6; see Fig. 1 legend) from adult mouse brain (Fig. 1c). During cerebellar development the antigen is faintly detectable in Western blots of a crude membrane fraction at embryonic day 15, in the form of two faint bands with apparent M_r s of 220K and 200K (Fig. 1g). With increasing age, immunoreactivity shifts from the 220K and 200K bands towards the 160K band (Fig. 1h–k). In the adult form the 220K component is no longer present (Fig. 1c, l). When J1 antigen is isolated preparatively from 'rest L2' of adult brain, the 200K component is not detectable, possibly because of degradation during purification procedures. The J1 antigen is a glycoprotein, as it can be biosynthetically labelled with fucose (Fig. 1m) and is recognized by monoclonal antibody L2 (result not shown). Cultured astrocytes (Fig. 1n), but not neurones (Fig. 1o) purified from early postnatal cerebellum, synthesize the 220K form; this component is also secreted into the culture supernatant. In mixed cerebellar cultures the antibody reacts strongly on immunofluorescence with glial fibrillary acidic protein (GFAP)-positive astrocytes, with O4 and O1 antigen-positive oligodendrocytes (Fig. 2) and with some fibronectin-positive fibroblast-like cells (not shown). Neurones acquire faint immunoreactivity with time in culture, possibly resulting from adsorption of the secreted 220K form of the antigen. As the J1 antigen is secreted into the culture medium, we examined its possible relationship to characterized extracellular matrix proteins. Radioimmunoassays, however, fail to detect any significant reaction of J1 antibody with laminin⁸, collagen IV⁹, fibronectin, nidogen¹⁰ or heparan sulphate proteoglycan¹¹ (results not shown).

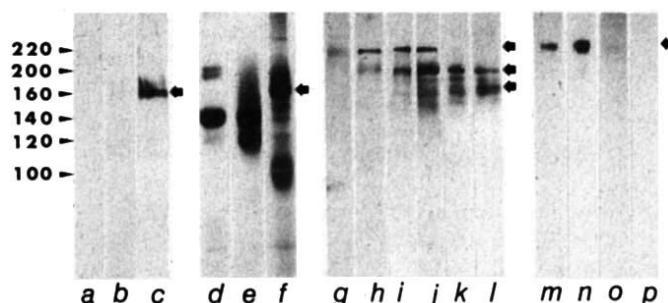
When adhesion of single-cell suspensions to monolayer cells is measured in the presence of Fab fragments of J1 antibody, neurone-astrocyte adhesion is inhibited, but not astrocyte-astrocyte or neurone-neurone adhesion (Table 1). The low level of adhesion between neurones and skin fibroblasts is also not disturbed to any measurable degree by J1 antibodies (not shown). That J1 antibodies do not interfere with adhesion of neurones to J1 antigen-positive skin fibroblasts could be the result of a molecular difference or a difference in cell-surface density of the J1 antigen between astrocytes and fibroblasts. Fab fragments of polyclonal antibodies to mouse liver membranes do not interfere with adhesion (Table 1), whereas polyclonal L1 antibodies prevent neurone-neurone adhesion, and polyclonal N-CAM antibodies prevent neurone-neurone, neurone-astrocyte and astrocyte-astrocyte adhesion (results not shown; see ref. 12).

The present experiments have revealed a novel neural cell adhesion molecule, the J1 antigen, which mediates neurone-astrocyte adhesion and may provide the molecular basis for the repeatedly observed preferential attachment of neuronal cell bodies to astrocytes, and the accelerated neurite extension on astrocyte membranes. As a secreted glycoprotein, J1 antigen may represent part of the extracellular matrix in the nervous system. As J1 antigen is synthesized by astrocytes and not by neurones, an adhesion mechanism between J1 glycoprotein(s) and a different receptor molecule(s) on neurones must be postulated. Whether L1 or N-CAM is involved in such ligand-receptor relationships, and whether J1 antigen contributes to the migration of granule cell neurones along the surface of Bergmann glial processes, remains to be seen.

J1 antigen is the third member of the L2/HNK-1 family of cell-surface glycoproteins to be examined for its involvement in

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Fig. 1 Immunochemical characterization of the J1 antigen. In Western blots, the J1 antibody did not react with immunopurified L1 (a) or N-CAM (b), and recognized the 160K component, but not the myelin-associated glycoprotein (MAG), the 100K component of 'rest L2' (c). Compare the Western blots (a-c) with the immunopurified L1 (d), N-CAM (e) and 'rest L2' (f) from adult mouse brain in reduced silver gels according to Merrill¹⁹. During cerebellar development, J1-immunoreactive material was examined by Western blot analysis of crude membrane fractions¹⁵ of C57BL/6J mice, after SDS-polyacrylamide gel electrophoretic separation on 6-12% gels with 200 µg protein per well, at embryonic day 15 (g), at postnatal days 0-2 (h), 4-6 (i), 8-12 (j) and 20-25 (k) and in the adult (>2 months old; l). m, J1 antigen is a glycoprotein, as it incorporates ³H-fucose into the 220K band; the lane shows an autoradiograph of an immunoprecipitate with J1 antibody. J1 antigen can be immunoprecipitated from cultures of pure astrocytes (>97% are positive for the astrocyte marker GFAP) as a strong 220K band (n) and as a very faint band from pure neurones (o) (>99% are positive for the neurone markers L1 antigen and tetanus toxin receptor) isolated from early postnatal mouse cerebellum as described elsewhere¹². The weak reactivity of the neuronal cultures is presumably caused by low contaminating levels of astrocytes and fibroblast-like cells. p, Autoradiograph of an immunoprecipitate from astrocytes reacted with preimmune rabbit antibodies. Arrows indicate the 160K, 200K and 220K components of J1 antigen.



Methods. Polyclonal antibodies were prepared in rabbits by injecting them with 160K glycoprotein isolated from adult mouse brain. The 160K band was purified from 'rest L2' (consisting of the L2 epitope-carrying molecules after removal of L1 antigen and N-CAM by immunoaffinity chromatography on monoclonal L1 and H28.123 (mouse N-CAM/BSP-2) columns⁶). 'Rest L2' was separated by chromatography on a Sephacryl S-300 column in Tris-buffered saline pH 8.2, containing 0.1% deoxycholate. The fractions containing the 160K glycoprotein were used for immunization. The pooled fractions were checked for purity in Western blots, which showed no reaction with polyclonal L1, N-CAM and MAG antibodies. On incubation with monoclonal L2 antibodies, the fractions showed mainly one broad band at 160K. This band was also the predominant one in SDS silver gels. Outbred rabbits were injected subcutaneously with 10 µg of purified 160K component in Tris-buffered saline, first in complete Freund's adjuvant and 2-3 weeks later in incomplete Freund's adjuvant. Seven days after the third intraperitoneal injection of 10 µg J1 antigen in Tris-buffered saline, rabbits were bled for serum. The purified IgG fraction of J1 antibodies was used in all experiments. ³H-fucose labelling was carried out in mixed cerebellar cultures¹⁶ from 6-day-old C57BL/6J mice (1×10^7 cells in a Petri dish of 6 cm diameter). After 3 days *in vitro*, cells were treated with ³H-fucose ($80 \mu\text{Ci ml}^{-1}$) in 1 ml of basal Eagle's medium containing 10% horse serum¹⁶ for 4 h in a CO₂ incubator, subsequently solubilized and used for immunoprecipitation as described previously¹². Immunoprecipitations of ³⁵S-methionine-labelled cells were carried out as described in detail elsewhere¹².

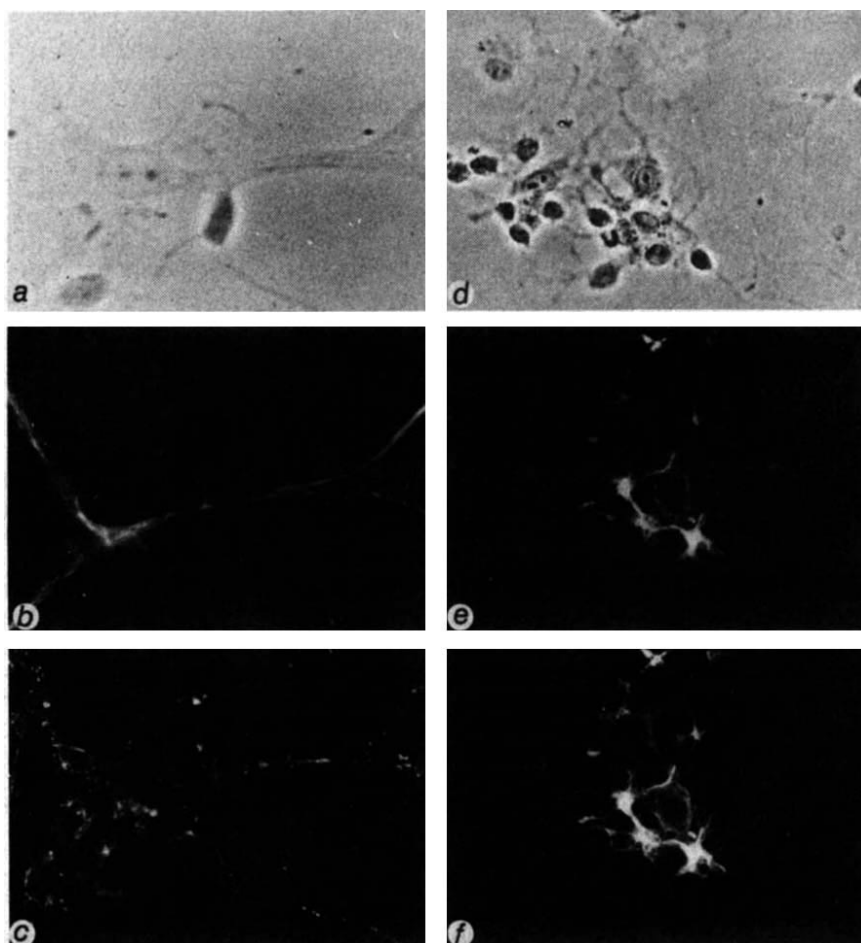


Fig. 2 Double immunofluorescence for J1 antigen (c,f) in monolayer cultures of 7-day-old C57BL/6J mouse cerebellum after 2 days *in vitro*; and for glial fibrillary acidic protein (b) and the oligodendrocyte marker O4 (e). a,d, Corresponding phase-contrast micrographs of the fluorescence images b,c and e,f, respectively. The procedures for cell culture and double immunofluorescence have been described elsewhere¹⁴⁻¹⁶. $\times 320$.

Table 1 Inhibition of adhesion between neurones and astrocytes from early postnatal mouse cerebellum in the presence of Fab fragments from J1 antibody

Antibody	Fab concentration (mg ml ⁻¹)	Neurone-neurone	Neurone-astrocyte	Astrocyte-neurone	Astrocyte-astrocyte
None	0	0 ± 3	0 ± 2	0 ± 4	0 ± 2
Polyclonal antibody to mouse liver	1	2 ± 4	-3 ± 2	-2 ± 3	1 ± 1
J1	1	-3 ± 8	47 ± 7	38 ± 15	-7 ± 12

The adhesion test was carried out as described elsewhere¹² using monolayer cultures as target cells and fluorescein diacetate-labelled single-cell suspensions as probe cells. Pure populations of astrocytes and neurones were obtained by buoyant density centrifugation of single-cell suspensions through Percoll, combined with immunocytolysis¹². Small cerebellar neurones were obtained from 6–8-day-old C57BL/6J mice and used as target cells after 3 days in culture, with over 99% of all cells expressing L1 antigen¹⁵ or tetanus toxin receptors¹⁶ and less than 1% expressing J1 antigen. Cerebellar astrocytes were obtained as described elsewhere¹² from 3–5-day-old mice by immunocytolysis with O4, MESA-1 and M5 antibodies in the presence of guinea pig complement, after 3 days of *in vitro* culture and subsequent subculture for 1 day; over 97% of all the cells expressed GFAP and more than 95% expressed J1 antigen. Fibroblasts were obtained from the skin of 1-day-old C57BL/6J mice by treatment with collagenase IV and trypsin¹⁷ and used as target cells after 3 days of *in vitro* culture. Over 90% of these cells expressed the J1 antigen. Probe cells were obtained from monolayer cultures of enriched neurones (after 1 day in primary culture), astrocytes (1 day after immunocytolysis and subsequent subculture) and fibroblasts (after 3 days in primary culture) by treatment with 20 µg ml⁻¹ trypsin, 1 mM EDTA in Ca²⁺, Mg²⁺-free Hank's balanced salt solution for 15 min at room temperature¹². Probe cells were labelled with fluorescein diacetate (10⁻⁶ M) during the trypsinization step. The mild trypsin treatment did not reduce the immunofluorescence staining intensities for either the J1 antibody or the antibody to mouse liver membrane¹⁸, as judged by comparison with non-trypsinized cells. Probe and target cells were treated with Fab fragments of J1 antibody for 20 min on ice before the adhesion test. Probe and target cells were then incubated for 30 min at room temperature in a reciprocal shaker at 40 cycles per min. Aggregation among probe cells was negligible in the presence or absence of Fab fragments. In the absence of Fab fragments the percentage of binding of total input cells was ~80% for neurone-neurone and neurone-astrocyte adhesion, 50% for astrocyte-astrocyte and astrocyte-neurone adhesion, and 10% for neurone-fibroblast adhesion (see ref. 12 for details). Per cent inhibition of adhesion in the presence of Fab fragments was calculated from:

$$\% \text{inhibition} = \left(\frac{\text{adhesion (control)} - \text{adhesion (+Fab)}}{\text{adhesion (control)}} \right) 100$$

Values are the mean of several experiments (±s.d.). Three experiments were performed for neurone-neurone adhesion, three for neurone-astrocyte adhesion, two for astrocyte-astrocyte, two for astrocyte-neurone and two for neurone-fibroblast adhesion. The difference in inhibition of adhesion due to liver membrane and J1 antibodies is significant for neurone-astrocyte and astrocyte-neurone adhesion ($P < 0.001$, Student's *t*-test).

cell-cell interactions, and indeed it has been shown to mediate adhesion. This result is in accord with our hypothesis that all L2/HNK-1-carrying molecules are functionally important in cell-surface interactions^{2,6}. The observation that further glycoproteins belong to the L2/HNK-1 family may now encourage and guide further search for new adhesion molecules displaying different sets of functional properties not only in the nervous system, but also in the immune system, where HNK-1 has been shown to react with a subclass of lymphocytes including natural killer cells¹³. The fact that all neural adhesion molecules identified so far share a carbohydrate moiety which is involved in adhesion¹², which appears to be developmentally regulated independently from the expression of the protein backbone of the adhesion molecule¹⁴, and which is differentially expressed on some, but not all N-CAM molecules⁶, indicates an important functional role¹² that is highly conserved among vertebrate species and which has yet to be elucidated in molecular terms.

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Pertussis toxin reverses adenosine inhibition of neuronal glutamate release

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Adenosine and its analogues are potent inhibitors of synaptic activity in the central and peripheral nervous system^{1,2}. In the central nervous system (CNS), this appears to arise primarily by inhibition of presynaptic release of transmitters^{3,4}, including glutamate⁵, which is possibly the major excitatory transmitter in the brain. In addition, postsynaptic effects of adenosine have been reported which would also serve to reduce neurotransmission^{6,7}. The mechanism by which adenosine inhibits CNS neurotransmission is unknown, although it appears to exert its effect via an A₁ receptor^{2,8} which in some systems is negatively coupled to adenylate cyclase⁹. In an attempt to elucidate the mechanism of inhibition, we have examined the effect of pertussis toxin (PTX) on the ability of the stable adenosine analogue (–)phenylisopropyladenosine (PIA) to inhibit glutamate release from cerebellar neurones maintained in primary culture. PTX, by ADP-ribosylating the nucleotide-binding protein N_i, prevents coupling of inhibitory receptors such as the A₁ receptor to adenylate cyclase¹⁰. As reported here, we found that PTX, as well as preventing inhibition of adenylate cyclase by PIA, also converts the PIA-induced inhibition of glutamate release to a stimulation. Our results suggest strongly that purinergic inhibitory modulation of transmitter release occurs by inhibition of adenylate cyclase.

Although previous experiments concerning the effects of adenosine on transmitter release from brain tissue have been performed on slices or synaptosomes, our preliminary results indicated that incubation of hippocampal slices (300 µm thick) for 1 h with PTX (0.1–1.0 µg ml⁻¹) had no effect on the ability of the adenosine analogue 2-chloroadenosine to inhibit trans-

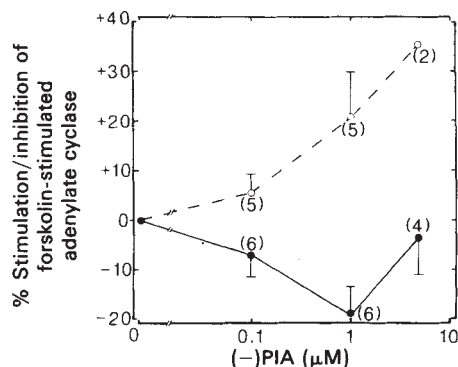


Fig. 1 Effect of incubation of cultured cerebellar neurones with PTX on $(-)$ PIA-induced inhibition of adenylyl cyclase in membranes prepared from the cells. We investigated the effect of $(-)$ PIA on the stimulation of adenylyl cyclase by a submaximal concentration of forskolin ($2 \mu\text{M}$). The basal adenylyl cyclase activity was 8.3 ± 3.5 and 8.9 ± 2.7 pmol per min per mg protein and the forskolin-stimulated activity was 14.7 ± 2.4 and 33.3 ± 8.8 pmol per min per mg protein in control (●) and PTX-treated (○) cells respectively (mean $\pm$ s.e.m.). Numbers in parentheses indicate number of experiments.

Methods. Rat cerebellar neurones were grown on poly-L-lysine in 35-mm Petri dishes for 13–21 days, having been treated with fluorodeoxyuridine ($80 \mu\text{M}$) after 2 days *in vitro* to reduce non-neuronal cell proliferation. They were incubated for 14–16 h at 37°C with either PTX (0.5 – $1.0 \mu\text{g ml}^{-1}$) or its vehicle (1 M NaCl , $10 \text{ mM Tris-HCl pH } 8$, diluted 100-fold) in 0.5 ml of growth medium (minimal essential medium (MEM), 10% fetal calf serum, 25 mM KCl). The cells were rinsed with buffer and collected in 10 mM Tris-HCl , $2 \text{ mM EGTA pH } 7.4$. After homogenization, the membranes were washed twice and adenylyl cyclase activity was assayed as described in ref. 13, except that EGTA (0.5 mM) was present in the final incubation medium and incubation was continued for 20 min.

mitter release (A.C.D. and E. R. Archer, unpublished data). As incubation of slices for longer periods markedly decreased the ability of the slices to synthesize and release glutamate, the effect of incubation with PTX for longer time periods was not examined. Instead, we examined the effects of PTX in a cell culture system. Cerebellar cells cultured from 3–6-day-old rats consist mainly of granule cells and have previously been found to release glutamate on depolarization, in a Ca^{2+} -dependent manner¹¹. The neurones were grown, as described previously^{11,12}, on coverslips for the transmitter release studies (for method see Fig. 2 legend). For each experiment, we determined the amount of newly synthesized ^3H -glutamate released in two periods of depolarizing stimulation (S_1 and S_2 ; see Fig. 2 legend). The S_2/S_1 ratio was then compared between experiments.

In adult animals, A_1 adenosine receptors have been shown to be associated with cerebellar granule cells¹³; in agreement with this observation, in our initial studies we found that inhibition of ^3H -glutamate release by $(-)$ PIA, which is a relatively selective A_1 agonist, developed at the same time as stimulus-dependent transmitter release became maximal, between 7 and 12 days *in vitro*, depending on the batch of neurones. For example, between days 7 and 9 *in vitro*, stimulation with $50 \mu\text{M}$ veratridine caused release of $1,553 \pm 332$ d.p.m. ($n=6$) over basal levels of ^3H -glutamate and $(-)$ PIA ($2 \mu\text{M}$) inhibited release by $12.1 \pm 1.2\%$ ($n=3$), whereas between days 14 and 21 *in vitro*, the same stimulus resulted in release of $2,860 \pm 713$ d.p.m. ($n=8$) of ^3H -glutamate and inhibition by $(-)$ PIA was $57.0 \pm 13.1\%$ ($n=4$). In subsequent experiments, neurones were used between days 13 and 21 *in vitro*.

When cultured neurones were incubated with PTX for 14–16 h, the inhibition of adenylyl cyclase by $(-)$ PIA seen in washed membranes prepared from a cell-free preparation of control cells (preincubated with vehicle) was converted to a marked stimulation (Fig. 1). This result not only indicates that in these cells PTX is effective in preventing negative coupling to adenylyl cyclase, in parallel with its effect in other sys-

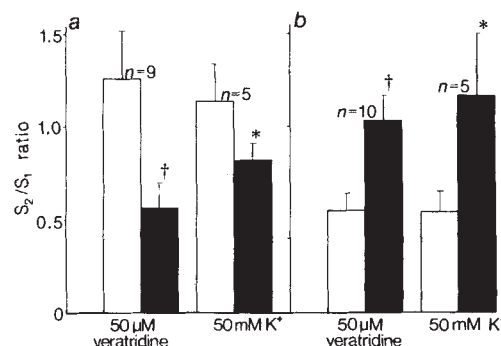


Fig. 2 Effect of PTX on the inhibition by $(-)$ PIA of ^3H -glutamate release from cultured cerebellar neurones. The amount of ^3H -glutamate released after stimulation was calculated by subtracting the averaged basal release, and the ratio S_2/S_1 was determined for each experiment. A pair of control (open columns) and $(-)$ PIA (solid columns) experiments were performed on the same day on the same batch of neurones, and the significances of the differences between their S_2/S_1 ratios (given as mean $\pm$ s.e.m.) were determined by paired t-tests. * $P < 0.05$; † $P < 0.01$. The outflow of ^3H -glutamate (in d.p.m.) during S_1 for control (a) and PTX-treated (b) neurones was, respectively, $4,899 \pm 1,452$ and $5,423 \pm 1,410$ for K^+ stimulation, and $1,979 \pm 264$ and $2,868 \pm 509$ for veratridine stimulation. The increases due to PTX treatment did not reach statistical significance.

Methods. Neurones (13–21 days *in vitro*), grown on 1-cm^2 coverslips, were preincubated for 14–16 h at 37°C with PTX (0.5 – $1.0 \mu\text{g ml}^{-1}$) or its vehicle in $100 \mu\text{l}$ growth medium. They were then rinsed and incubated with ^3H -glutamine ($5 \mu\text{Ci}$ in $100 \mu\text{l}$ of Krebs-Ringer buffer (KRB) $\text{pH } 7.4$; 320 mM) for 45 min. The coverslips were rinsed and transferred to a perfusion chamber¹² and perfused with KRB at 35°C at a rate of 1 ml min^{-1} . Two periods of stimulation were applied 18 min (S_1) and 36 min (S_2) after the start of perfusion, with either 50 mM K^+ -KRB for 4 min or $50 \mu\text{M}$ veratridine-KRB for 3 min. To examine the effect of $(-)$ PIA ($2 \mu\text{M}$), it was included in the KRB from 27 min after the start of perfusion. Fractions collected at 1-min intervals were placed in tubes containing 10 mM each of carrier glutamine, glutamate and γ -aminobutyric acid (GABA) and 1.5 nCi of ^{14}C -glutamate to correct for recovery. The ^3H -glutamate in the fractions was separated from labelled glutamine and GABA by column chromatography as described previously⁵.

tems^{14,15}, but also that PTX treatment allows the expression of an underlying stimulation of adenylyl cyclase, possibly via A_2 receptor activation.

We then examined the effect of PTX on the ability of $(-)$ PIA to inhibit depolarization-induced release of newly synthesized glutamate. In control experiments in the absence of $(-)$ PIA, PTX altered the pattern of release of ^3H -glutamate, in that the S_2/S_1 ratio was reduced in PTX-treated compared with control cells (Fig. 2). It is possible that the enhanced release of ^3H -glutamate in S_1 relative to S_2 in PTX-treated cells results from either an increased concentration of endogenous cyclic AMP or the inability of endogenously released adenosine to inhibit glutamate release in PTX-treated cells. Because of the finite amount of ^3H -glutamate available for release, there would be a corresponding reduction in S_2 and thus a reduction in the S_2/S_1 ratio.

We observed a more marked effect of PTX treatment on the response to PIA. Whereas in control cells $(-)$ PIA ($2 \mu\text{M}$) inhibited both veratridine- and K^+ -stimulated release of ^3H -glutamate by 55 and 28%, respectively (Fig. 2a), in PTX-treated cells there was a marked enhancement of ^3H -glutamate release in the presence of $(-)$ PIA (Fig. 2b), by 87 and 117% for veratridine and K^+ stimulation, respectively.

The conversion of $(-)$ PIA-induced inhibition of ^3H -glutamate release to a stimulation by pretreatment with PTX mirrors the effect of PTX on adenylyl cyclase in these cells. In addition, in control intact cerebellar neurones, synthesis of cyclic AMP is enhanced by the depolarizing stimuli used, and PIA inhibits both control and K^+ -stimulated cyclic AMP production (A.C.D. and S.A.P., preliminary results). We suggest, therefore, that the

mechanism by which adenosine analogues inhibit presynaptic neuronal activity involves inhibition of adenylate cyclase. Furthermore, stimulation of adenylate cyclase by (-)PIA in PTX-treated cells may be responsible for the observed enhancement of transmitter release. Previous studies have suggested that selective A₂ receptor activation and augmented cyclic AMP levels enhance transmitter release^{5,15}.

The ionic mechanism associated with inhibition of synaptic activity by adenosine analogues acting at A₁ receptors remains a matter for speculation. Adenosine analogues have been found to inhibit synaptosomal ⁴⁵Ca fluxes¹⁶, whereas in an electrophysiological study they had no apparent effect on voltage-sensitive Ca²⁺ currents in hippocampal CA1 cells¹⁷. However, in neuroblastoma¹⁸ and rat dorsal root ganglion cells¹⁹, Ca²⁺ currents were potentially inhibited by adenosine analogues. It is possible that different mechanisms exist for pre- and postsynaptic effects of A₁ receptor stimulation.

In several systems PTX has been used to distinguish between receptors which are negatively coupled to adenylate cyclase, and those which are termed calcium-mobilizing, on which PTX had no effect^{20,21}. However, in mast cells PTX has been reported to block the secretory process by preventing activation of calcium-mobilizing receptors²². As neither A₁ nor A₂ adenosine receptors have been classified in this way²³, and as in the present study PTX enhanced rather than inhibited transmitter release, it is unlikely that it acts on calcium-mobilizing receptors. Thus, the results presented here provide clear evidence for the involvement of the N₁ protein in the inhibitory effect of A₁ receptor stimulation on transmitter release; it can thus be classed in the same functional group of presynaptic neuronal receptors as enkephalin and α₂ receptors²⁴.

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Activated human eosinophils generate SRS-A leukotrienes following IgG-dependent stimulation

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Eosinophils, a class of granular leukocytes, are prominent in many inflammatory processes, particularly in asthma, certain allergic diseases and during infections with helminthic parasites. Following incubation with the Ca ionophore A23187 (refs 1–4) (a non-physiological agent which circumvents membrane calcium-gating mechanisms⁵), eosinophils generate large amounts of sulphido-peptide leukotrienes, potent inducers of smooth muscle constriction^{6–9} and mucus production¹⁰. These are now known to represent the activity previously termed 'slow-reacting substance of anaphylaxis' (SRS-A) but attempts to identify a physiological stimulus for SRS-A production by eosinophils have so far been unsuccessful. The cells contain recognized receptors for IgG (Fc)^{11–14} and it is known that they adhere to, and can be activated by, contact with the surface of large organisms such as helminthic larvae¹⁵. We show here that eosinophils, particularly when activated, produce sulphido-peptide leukotrienes after contact with large particles coated with IgG.

We found that normal eosinophils generated appreciable amounts of SRS-A leukotrienes when incubated with IgG-coated particles, and that this was enhanced twofold when cells were stimulated *in vitro* (by the bacterial analogue f-Met-Leu-Phe (fMLP)) and up to 10-fold when activated low-density cells (from patients with hypereosinophilia) were studied.

Evidence for a harmful role for eosinophils in asthma is

provided by the observation that one of the granular constituents, major basic protein, is released onto and damages bronchial epithelium^{16–19}. On the other hand, this mechanism might give the eosinophil a protective function in adaptive immunity to certain parasitic diseases^{20,21}.

A23187-dependent eosinophil generation of sulphido-peptide leukotrienes (LTC₄, LTD₄, LTE₄)^{1–4} provides a theoretical mechanism by which eosinophils could contribute to the immediate asthmatic reaction. However, the physiological relevance of this observation has remained uncertain because of the artificial nature of the stimulus. Despite evidence of generation of leukotrienes from other circulating cell types using more pathophysiological agents (that is, LTB₄ from neutrophils by opsonized zymosan²², LTB₄ and LTC₄ from mononuclear cells by unopsonized zymosan²³ and LTC₄ from alveolar macrophages by IgE complexes²⁴), no comparable trigger for eosinophil sulphido-peptide leukotriene generation has been identified.

Eosinophils from patients with hypereosinophilia have an increased capacity to kill helminths²⁵. Many of these cells are hypogranular and have reduced density^{26–28}. These low-density cells have altered oxidative metabolism^{29,30}, an enhanced capacity to kill schistosomula of *Schistosoma mansoni in vitro*³¹ and increased expression of IgE (Fc) receptors^{32,33}. In addition, low-density eosinophils have an altered expression of cell-surface antigenic determinants³⁴ and are thought to be a sub-population of 'activated' cells.

In the present study IgG-coated Sepharose beads, which are 5–10 times larger than eosinophils (50–100 μm in diameter compared with 14 μm), were used as a stimulant, and the capacity of low-density and normal-density eosinophils to generate sulphido-peptide leukotrienes was examined. Incubation of normal-density eosinophils with IgG-coated beads resulted in a time-dependent release of these leukotrienes (Fig. 1b) related to the amount of adherent IgG (Fig. 1a) and not found with beads coated with human albumin. Furthermore, leukotriene generation by eosinophils in the presence of IgG-coated beads was significantly enhanced by prior incubation with fMLP (10⁻⁸ M) for 30 min (*P* < 0.02) (Fig. 1a, b). Incubation with fMLP in the

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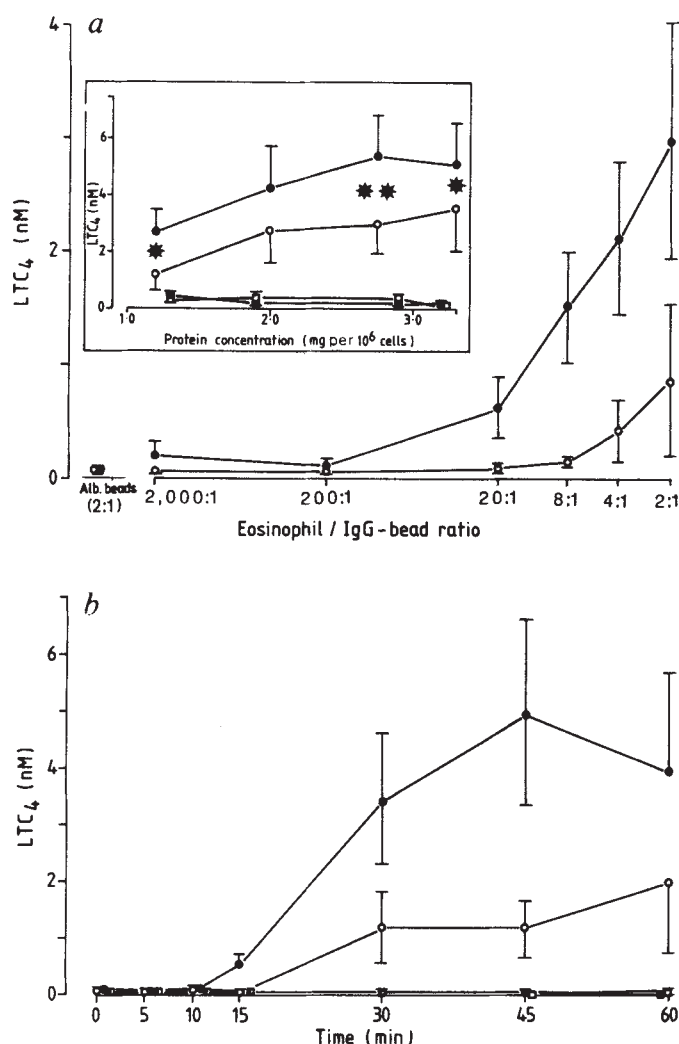
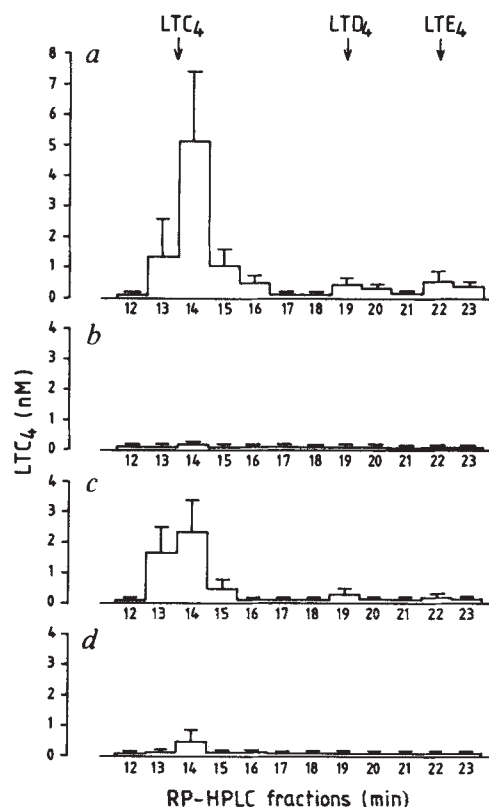


Fig. 2 Immunoreactive LTC₄ in the reverse-phase HPLC fractions of the eosinophil products from incubation of: *a*, low-density cells with IgG-coated beads; *b*, low-density cells with albumin-coated beads; *c*, normal-density cells with IgG-coated beads; *d*, normal-density cells with albumin-coated beads (mean $\pm$ s.e.m., $n=3$), were compared with the retention times of synthetic standards.

Methods. Immunoreactive LTC₄ in the reverse-phase (RP) HPLC fractions of supernatants from eosinophils incubated with IgG-coated beads. Eosinophils (10^6 ml⁻¹) were incubated with protein-coated beads as described in Fig. 1 legend. The leukotrienes in the supernatant were extracted in 80% methanol which was diluted to 10% with water and acidified to pH 4 before further extraction using a C18 Sep-Pak (Waters Associates)^{3,44}. The leukotrienes eluting in 100% methanol were evaporated to dryness, resuspended in running buffer (methanol/water/acetic acid, 70:30:0.1, adjusted to pH 5.4 with ammonia) and separated by RP-HPLC on a Nucleosil C18 column. The fractions were evaporated to dryness and resuspended before radioimmunoassay.

Fig. 1 *a*, Generation of LTC₄ by eosinophils in response to incubation with varying numbers of IgG-coated Sepharose beads with or without prior incubation with fMLP. Normal-density eosinophils (10^6 ml⁻¹, >90% purity) were incubated for 30 min with fMLP (10^{-8} M) (●) or buffer (○) followed by 30 min with varying numbers of IgG-coated beads (2.75 mg IgG per 5×10^5 beads) or albumin-coated beads (2.9 mg albumin per 5×10^5 beads) (mean $\pm$ s.e.m., $n=4$). Inset, effect of fMLP on LTC₄ generation by eosinophils incubated with IgG-coated beads. Normal-density cells (10^6 ml⁻¹, >90% purity) were incubated with fMLP or buffer for 30 min followed by 30 min with Sepharose beads (5×10^5 ml⁻¹) coated with varying doses of IgG or albumin. ●, fMLP + IgG-coated beads; ○, buffer + IgG-coated beads; ■, fMLP + albumin-coated beads; □, buffer + albumin-coated beads (mean $\pm$ s.e.m., $n=8$, * $P<0.05$, ** $P<0.02$). *b*, Time course of release of LTC₄ by eosinophils incubated with IgG-coated beads with enhancement by fMLP. Normal-density eosinophils (10^6 ml⁻¹, >90% purity) were incubated for 30 min with fMLP (10^{-8} M) or buffer, followed by addition of either IgG- (2.75 mg ml⁻¹) or albumin- (2.9 mg ml⁻¹) coated beads (5×10^5 ml⁻¹) and the incubation terminated after varying time intervals. Symbols, ●, fMLP + IgG-coated beads; ○, buffer + IgG-coated beads; ■, fMLP + albumin-coated beads; □, buffer + albumin-coated beads (mean $\pm$ s.e.m., $n=4$).

Methods. Human eosinophils were purified on discontinuous metrizamide gradients⁴¹. Populations were identified on the basis of density, normal density (>1.12 g ml⁻¹) in fractions 4 and 5 and low-density cells (<1.12 g ml⁻¹) in fractions 2 and 3. The eosinophils (10^6 ml⁻¹) were incubated with either fMLP (10^{-8}) or buffer at 37 °C for 30 min. The cells were further incubated with human IgG- or human albumin-coated beads, which were left to sediment during the course of the incubation. Beads were prepared with cyanogen bromide-activated Sepharose 4B particles (Pharmacia). These particles were swollen in 1 mM HCl and washed with coupling buffer (0.2 M NaHCO₃, pH 8.7 containing 0.5 M NaCl). Aliquots (1 ml) of gel were incubated with 10, 7.5, 5 and 2.5 mg of human IgG or albumin (Sigma), dissolved in coupling buffer, for 2 h at room temperature with continuous mixing. The purity of the IgG preparation was determined by crossed immunoelectrophoresis (kindly performed by Dr Joan Longbottom). The preparation contained >98% IgG, with albumin and pre-albumin the principal contaminants. There was <0.001% IgE as assessed by a double-antibody radioimmunoassay⁴² (kindly performed by Dr T. G. Merrett). Any remaining reactive groups on the Sepharose were blocked by incubating particles with 1 M ethanolamine pH 8.0 for 2 h at room temperature with frequent mixing. The particles were washed alternately with coupling buffer and acetate buffer (0.1 M, pH 4.0, containing 0.5 M NaCl) to remove any noncovalently bound protein. This resulted in preparations containing 3.30, 2.75, 2.00 and 1.20 mg IgG per 5×10^5 beads, respectively, determined after alkaline digestion of the coupled beads. The incubation was terminated by centrifugation (8,000g for 1 min) and the supernatant stored at -80 °C before radioimmunoassay. Samples were assayed in duplicate using a specific sulphidopeptide leukotriene radioimmunoassay using antisera raised in rabbits which gave cross-reactivity between LTC₄, LTD₄ and LTE₄ of 100:70:8 (refs 3, 43, 44). Statistical analysis was performed using the Wilcoxon matched-pair signed rank test.



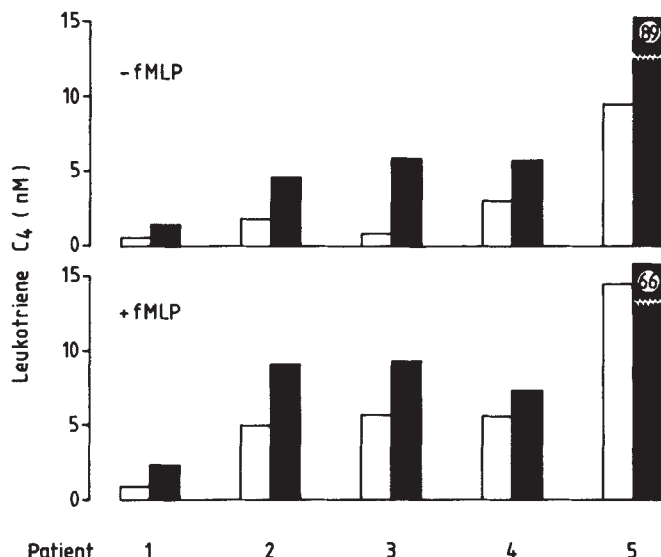


Fig. 3 LTC₄ generation by low-density and normal-density eosinophils incubated with IgG-coated beads with or without fMLP. The eosinophils were collected from four patients with hypereosinophilic syndrome and one with Churg-Strauss hypereosinophilia (patient 5). The cells were separated on metrizamide gradients into low density (metrizamide fractions 2 and 3, density <1.12 g ml⁻¹) and normal density (metrizamide fractions 4 and 5, density >1.12 g ml⁻¹). The cellular constituents of the low-density fraction from each of the patients were: patient 1, 67% eosinophils (E), 30% neutrophils (N), 30% mononuclear cells (M); patient 2, E, 38%; N, 54%; M, 8%; patient 3, E, 90%; N, 10%; M, 0%; patient 4, E, 54%; N, 46%; M, 0%; patient 5, E, 92%; N, 0%; M, 8%. The cellular constituents of fractions 4 and 5 were: patient 1, E, 95%; N, 5%; patient 2, E, 90%; N, 10%; patient 3, E, 94%; N, 6%; patient 4, E, 95%; N, 5%; patient 5, E, 91%; N, 9%. LTC₄ concentrations (mean $\pm$ s.e.m., $n=5$) were measured after incubating the low-density and normal-density eosinophils (10⁶ ml⁻¹) for 30 min with fMLP (10⁻⁸ M) followed by IgG-coated (black columns) or albumin-coated (white columns) beads (5 $\times$ 10⁵ ml⁻¹) at protein concentrations as shown ($\pm$ 0.1 mg).

presence of albumin-coated beads was not associated with leukotriene generation. The leukotriene production by eosinophils was dependent on the number of beads present, with a maximal eosinophil-to-bead ratio of 2:1 (Fig. 1a).

The identities of the sulphidopeptide leukotrienes produced at 30 min by both normal- and low-density cells were confirmed by comparing immunoreactivity of the fractions with the retention times of synthetic markers using reverse-phase HPLC (Fig. 2). Both cell types generated LTC₄ following incubation with IgG-coated beads. A small amount of activity was present in fractions co-eluting with synthetic LTD₄, indicating partial degradation of LTC₄ to LTD₄. No sulphidopeptide leukotriene immunoreactivity was identified in the products of eosinophils incubated with albumin-coated beads.

LTC₄ generation in the presence of IgG-coated beads by low-density and normal-density eosinophils from five patients with eosinophilia was compared (Fig. 3). In each case the low-density cells generated greater amounts of LTC₄ than the normal-density cells from the same patients over a dose range of IgG. This increased production varied from 1.5 to 10 times that of the normal-density cells and was related to the proportion of eosinophils in metrizamide fractions 2 and 3. Neither normal- nor low-density cells generated LTC₄ when incubated with albumin-coated beads. Production of LTC₄ by low-density cells with IgG-coated beads (16 ng per 10⁶ eosinophils) was less than that of normal-density cells treated with Ca ionophore A23187 (41 ng per 10⁶ eosinophils)³; only a slight increase in LTC₄ generation was noted following preincubation of low-density cells with fMLP.

Confirmation that the LTC₄ originated from the normal- and low-density eosinophils was obtained by showing that

neutrophils failed to produce LTC₄, and that a mononuclear cell fraction, which also contained basophils (1.2%), generated <1.5 ng LTC₄ per 10⁶ cells. We assumed cell viability in all experiments, as leukotrienes are not preformed and their synthesis following stimulation requires energy and is dependent on cell integrity.

The stimulus in this study was IgG (Fc), known to bind to eosinophils by specific Fc receptors¹¹⁻¹⁴. It has been shown previously that fMLP and other chemotactic factors enhance the expression of IgG (Fc) receptors on eosinophils, suggesting that the increased LTC₄ production observed here results from greater stimulus-response coupling^{35,36}. IgG aggregates alone did not consistently trigger LTC₄ generation by eosinophils. On the other hand, the beads provide a large surface similar to that which occurs in helminth cytotoxicity *in vitro*, in which eosinophil secretory activity is stimulated^{15,37}.

The demonstration of LTC₄ generation by activated human eosinophils in response to stimulation with IgG-coated beads may have important implications for human disease. Eosinophils involved in gastrointestinal helminthic infections³⁸ would have access to IgG-coated worms and the resultant leukotriene generation causing mucous hypersecretion and smooth muscle contraction might favour worm expulsion. Similarly, in allergies (where IgG antibodies accompany the IgE response³⁹) eosinophil-derived leukotrienes might amplify the mucus production, bronchospasm, hyperaemia and oedema associated with these conditions. Comparable amounts of LTC₄ have been found in body fluids during allergic reactions (for example, 20 pmol LTC₄ extracted from nasal secretions following allergen challenge⁴⁰).

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A group of genes controlling the spatial expression of the bithorax complex in *Drosophila*

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The morphological diversity among the body segments of *Drosophila*, as displayed in the pattern of the larval cuticle¹, arises during embryogenesis by the selective expression of homoeotic genes. For example, the bithorax complex (BX-C) confers abdominal development on the segments in the posterior half of the embryo which otherwise would develop into thoracic segments². Mutations in trans-regulatory genes such as *Polycomb* (*Pc*)² or *extra sex combs* (*esc*)³ cause indiscriminate expression of the BX-C genes, which leads to all body segments being abdominal. I report here on mutations in four other trans-regulatory genes, members of a novel class of more than 20 genes controlling the spatial expression of BX-C genes. In contrast to *Pc* and *esc* mutations, only partial transformation into posterior abdominal development is observed in mutant embryos. However, embryos mutant for two or more of these genes show strong homoeotic transformation of all body segments similar to, or stronger than, that seen in *Pc* and *esc* embryos, indicating that these genes act synergistically in normal development to control the spatial expression of the BX-C genes.

Recent large-scale screens both for embryonic-lethal mutations affecting the pattern of the larval cuticle⁴⁻⁶ and for dominant 'extra sex combs' mutations (G.J., manuscript in preparation), yielded zygotic mutations in loci other than *Pc* which resemble weak *Pc* mutations in both their dominant adult and recessive embryonic phenotypes. These genes will be collectively referred to as the *Pc* group after its first known and most prominent member. Some of the mutations in the four *Pc* group loci described below were isolated for their embryonic head defect phenotypes, while most of them were identified on the basis of their dominant 'extra sex combs' trait in adult flies. The penetrance of this dominant phenotype depends on culture conditions and genetic background, but the recessive phenotype is embryonic lethality associated with an incomplete transformation of several body segments into more posterior abdominal segments. Three of these genes have not been identified previously, whereas one of them (*Polycomb-like Pcl*) appears to be identical to that described by Duncan⁷ (Table 1).

Mutations at the *Pcl* and *Sex comb on midleg* (*Scm*) loci cause posteriorly directed transformation of abdominal segments in the embryo. In the strongest alleles available, the first abdominal segment resembles the second abdominal segment and abdominal segments 2-7 are transformed into more posterior abdominal segments, while the head and thorax are normal (Fig. 1b). Weaker alleles affect only the more posterior segments, while the weakest *Pcl* allele is homozygous viable and transforms all the legs of adult flies to forelegs, including complete sex combs in males. This phenotype, which resembles the adult phenotype associated with mutations in *Pc* and other putative trans-regulatory genes^{3,7-10}, presumably results from the abnormal expression in the posterior leg disks of the *Sex combs reduced* gene, a member of the *Antennapedia* complex¹¹. Mutations at the *Posterior sex combs* (*Psc*) and *Additional sex combs* (*Asx*) loci represent a different class of phenotype in that they affect the entire body pattern of the embryo. The most prominent feature is a head defect while the posteriorly directed transformation is rather subtle in both head and thorax but clear in the abdomen (Fig. 1c). The incomplete homoeotic transformations associated with the *Pc* group mutations are apparently not the result of incomplete inactivation of these genes as the strongest alleles in *trans* to their corresponding deficiencies (*Pcl*, *Scm*) or the homozygous deficiencies (*Psc*, *Asx*) cause essentially the same phenotypes (Table 1): It should be noted that the lack-of-function phenotypes may be stronger than the amorphic phenotypes described here if these genes were also expressed during oogenesis.

Embryos which are homozygous mutant for any pair of the four *Pc* group loci show strong posteriorly directed transformation of the entire body pattern, resembling the amorphic *Pc* phenotype (Fig. 1d, e). The denticle bands of the trunk segments look very similar to one another and resemble the normal last abdominal denticle band. The head does not undergo involution and two abdominal denticle bands appear there, one ventrally in the gnathal region between the prothorax and the pharynx, and another on the dorsal surface of the head. Although *Pcl* and *Scm* individually appear to affect only the abdomen, the strong transformation seen in double mutant embryos (Fig. 1d) indicates that the *Pcl* and *Scm* gene functions are required for normal development not only in the posterior but also in the anterior half of the embryo.

The embryonic phenotype of the triple mutant *Psc Asx Pcl* is characterized by a tandem array of posterior abdominal segments including up to four abdominal denticle bands in the head region (Fig. 2a). If this transformation were caused by indiscriminate expression of BX-C genes, it should be suppressed in embryos which also lack the BX-C genes as is the

Table 1 Genetic and phenotypic features of *Pc* group genes

Locus	Map position	Cytology*	No. of alleles†	Embryonic phenotype‡
<i>Additional sex combs</i> (<i>Asx</i>)	2-72	51AB	6 (3)	H,T to (A); A1-7 to Ap
<i>Polycomb-like</i> (<i>Pcl</i>)§	2-83	55AF	8 (5)	A1 to (A2); A2-7 to Ap
<i>Posterior sex combs</i> (<i>Psc</i>)	2-67	49EF	1	H,T to (A); A1-7 to Ap
<i>Sex comb on midleg</i> (<i>Scm</i>)	3-49	85EF	4 (2)	A1 to (A2); A2-7 to Ap

* Assignments to polytene chromosome regions are based on the inclusion of the locus in a deficiency: *Asx* in *Df(2)L⁴⁴⁸*; *Pcl* in *Df(22)PC4*; *Psc* in *Df(2R)vg^D* and *Df(2R)vg^B* but not in *Df(2R)vg^C* (ref. 4); *Scm* in *Df(3R)by¹⁰* and *Df(3R)by⁶²* (ref. 13) but in neither *Df(3R)roe^{XMA}* nor the deficiency segregated by T(2;3)Ctx (ref. 5).

† Two mutants, one *Asx* allele (IIF51) and the *Psc* allele (IIN48), were isolated as embryonic lethal mutations causing head defects⁴. Two *Pcl* alleles (E33 and E90) were isolated as dominant extra sex combs mutations by G. Struhl. One *Asx* allele (P2) was isolated as a dominant enhancer of *Pc* by Dura *et al.*⁸. All other mutants were isolated on the basis of their dominant 'extra sex combs' phenotype in the F₁ male progeny of either ethyl methane sulphonate-treated (50 mM, 24 h) or X-irradiated (4,000 R, 100 kV, 10 mA, 1 mm A1 filter, 350 R min⁻¹, Philips MG102) males of one of the following genotypes: *b pr cn sca*, *b pr cn wtx bw*, *st e*, *cp in ri p^o* (ref. 14). Numbers of weak alleles are given in parenthesis. Classification of alleles as either strong or weak is based on the strength of the embryonic phenotype which, however, varies depending on the genetic background. So weak and strong alleles at any of the four loci may show very similar phenotypes, except *Pcl^{XT56}*, which is homozygous viable.

‡ Amorphic or probably amorphic phenotypes: H,T to (A), abdominal denticles in head and thorax; A1 to (A2), incomplete transformation of abdominal segment 1 into segment 2; A1-7 or A2-7 to Ap, abdominal segments 1-7 or 2-7 transformed to more posterior segments, often in a graded fashion with segments 5-7 closely resembling A8.

§ *Pcl* is probably identical to the *Pcl* locus described by Duncan⁷ as judged by map position, cytology and similarity of phenotype.

|| Chao-ting Wu (personal communication) has found that alleles of *Suppressor of zeste-2* do not complement the lethality of *Psc^{IIN48}*. However, the three alleles 1, 4 and 5 of *Su(z)2* do not share the embryonic phenotype of *Psc^{IIN48}* and *vg^D* (unpublished data) so the allelic relationship between *Psc* and *Su(z)2* should be considered unclear.

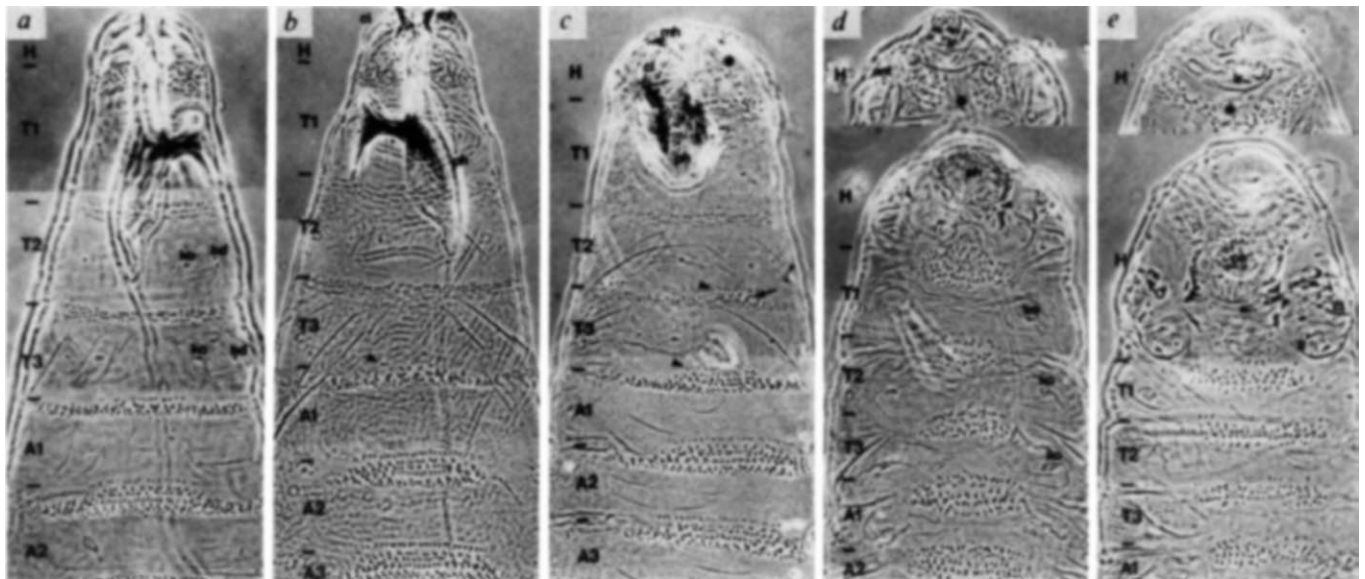


Fig. 1 Ventral aspects of the anterior halves of wild-type (*a*) and *Pc* group mutant (*b-e*) embryos. Eggs were collected and processed for microscopic examination as described in ref. 4 but with the following modifications. After dechorionation the vitelline membrane was removed by pricking the eggs at the posterior pole with a sharpened glass needle. After fixation in glycerol/acetic acid (1:1) at 60 °C for 15 min, the embryos were mounted in Hoyer's medium¹⁵ lactic acid (1:1). Phase contrast optics, Zeiss immersion objective ($\times 25$). *a*, The ventral cuticle pattern of wild-type embryos is characterized by three thoracic and eight abdominal denticle bands which mark the anterior margins of the segments. The segments can be morphologically distinguished on the basis of the shape of the denticle band, the number and orientation of denticle rows, and the size and pigmentation of denticles. For example, abdominal segments A2-A8 but not A1 bear first and fourth rows of anteriorly pointing denticles. Special sensory structures, the Keilin's organs and the black dots, are unique to the three thoracic segments. For a detailed description of the cuticle pattern of the wild-type larva, see ref. 1. *b*, *Pcl* embryos have a normal head and thorax but the abdominal segments are changed. A1 is partially transformed into A2 as evidenced by the incipient first row of anteriorly pointing denticles (arrowhead) and the blunter shape of the denticle band. Segments A2-A7 resemble more posterior segments of wild-type embryos. *Scm* embryos show essentially the same phenotype as *Pcl* embryos. *c*, *Asx* embryos do not complete head involution. Abdominal denticles are found anterior to T1 (asterisk) and in thoracic denticle bands (arrow). The first rows of abdominal denticles (arrowheads) are present in T3 and A1, indicating partial transformation into A2. Segments A2-A7 resemble more posterior segments of wild-type embryos. *Psc* embryos show very similar phenotypic features. *d*, In the *Pcl;Scm* double mutant, all denticle bands are similar to one another and resemble A8 of wild-type embryos, although the thorax appears less transformed as the Keilin's organs are present. Head involution does not occur so the remnants of most of the head structures are visible on the outside. A prominent abdominal denticle band appears on the dorsal surface of the head (asterisk) and a few abdominal denticles are found behind the floor of the pharynx (arrowhead). *e*, The overall pattern of the *Psc;Asx* double mutant closely resembles the phenotype shown in *d* but the shape of the denticle bands differs slightly. In addition to the abdominal denticle band on the dorsal surface of the head (asterisk), abdominal denticles appear behind the floor of the pharynx (arrowheads). Abbreviations: H, head; T1-3, pro-, meso- and metathoracic segments; A1-3, abdominal segments 1 to 3; ant, antennal sense organ; bd, black dots; ci, cirri; L, ko, Keilin's organ; li, labial sense organ; lr, labrum; mh, mouth hooks; ph, floor of pharynx.

case with *Pc* BX-C⁻ and *esc*; BX-C⁻ embryos^{2,3}. Indeed, no abdominal features are observed in *Psc* *Asx* *Pcl*; BX-C⁻ embryos (Fig. 2*b*), so functional BX-C genes are also required for the transformation in *Psc* *Asx* *Pcl*. There are additional phenotypic differences between *Psc* *Asx* *Pcl*; BX-C⁻ and BX-C⁻ embryos (Fig. 2*c*) which indicate that the expression of other homoeotic genes is also altered. Mesothoracic denticle bands are found not only in the mesothorax to abdominal segment 7 but also in the prothorax and in abdominal segment 8. As in *esc*; BX-C⁻ embryos³, another mesothoracic denticle band appears on the dorsal surface of the head. Other features are apparently unique to *Psc* *Asx* *Pcl*; BX-C⁻ embryos. Chitinous structures similar to those behind the last abdominal denticle band of BX-C⁻ embryos (Fig. 2*c*) occur in most segments, and abdominal segment 9 bears a prothoracic denticle band (Fig. 2*b*). This phenotypic effect can be interpreted as incomplete transformation of trunk segments towards head structures, which may result from partial inactivation of the *Antennapedia* (*Antp*) and *Scr* genes in segments T1 to A7, as suggested by comparison with the *esc*; *ScrAntp* BX-C⁻ embryonic phenotype¹⁷.

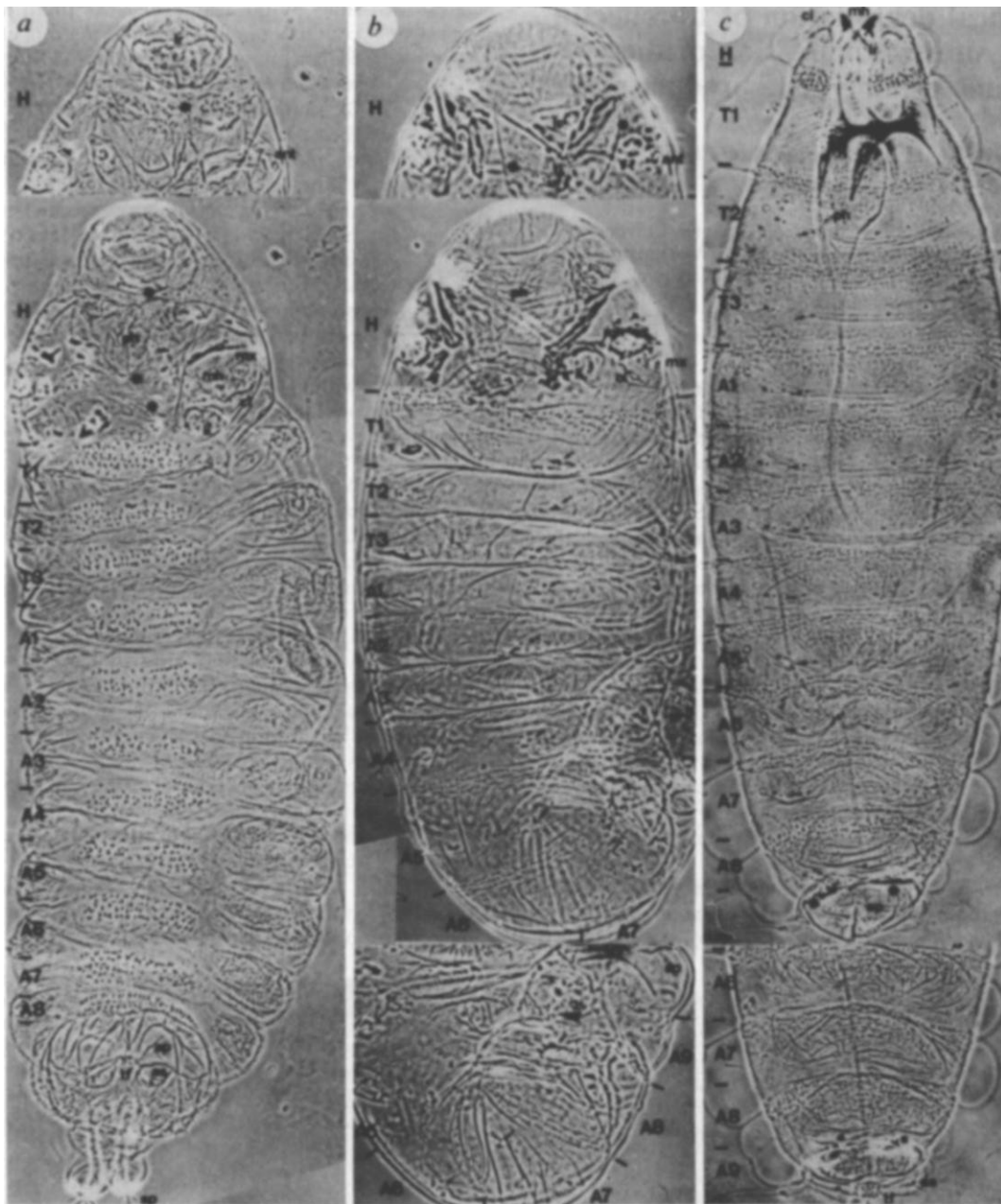
The results reported here show that the four *Pc* group genes *Psc*, *Asx*, *Pcl* and *Scm* act as *trans*-regulatory elements of BX-C expression by the same phenotypic criteria as applied to *Pc* and *esc*. However, at least two *Pc* group genes must be mutant to elicit the same degree of homoeotic transformation as *Pc* and *esc* mutations. As the *Pc* group genes are functionally related to one another and yet each makes a different contribution to the spatial control of BX-C expression, their relationship is reminiscent of genes which arose during evolution by gene

duplication and subsequently diverged functionally. If the *Pc* group provides an integrated system of control for the spatial expression of homoeotic genes, the lack of a single component destabilizes the system, but only the loss of another component causes its breakdown.

The approach used here to demonstrate the role of the four *Pc* group genes in the spatial control of BX-C expression provides a more sensitive and direct means of identifying *trans*-regulatory elements than the adult phenotype scored previously^{2,3,7-10}. The strong homoeotic transformation seen in double mutant embryos has led to the identification of 10 *Pc* group genes whose mutant alleles were originally isolated for their embryonic head defects but do not show the dominant 'extra sex combs' phenotype and hence would have escaped detection in previous screens (G.J., in preparation).

The strong embryonic transformation caused by *Pc* group double mutants has been used as an assay for the occurrence of additional *Pc* group genes in available deficiencies covering ~20% of the genome (refs 4-6). When these deficiencies are combined with mutations in one of the four genes described here, seven deficiency regions are found to cause strong embryonic transformation, suggesting the existence of ~40 *Pc* group genes in the entire genome of *Drosophila* (G.J., in preparation). In addition, 7 of 18 second-chromosomal *Pc* group genes identified by point mutations are uncovered by deficiencies representing ~30% of the second chromosome; this value indicates that nearly all the *Pc* group genes on the second chromosome that can be detected with the double mutant assay have been identified. The estimated number of about 40 zygotically

Fig. 2 Comparison between *Psc Asx Pcl* (a), *Psc Asx Pcl;P9* (b) and *P9* (c) embryos. *P9* deletes the entire BX-C^{2,16}, causing thoracic transformation of abdominal denticle bands and resulting in the presence of chitinous structures posterior to transformed segment A8^{2,17}. a, Extreme transformation of all body segments towards A8. Abdominal denticle bands appear in the head region at four different levels, one on the dorsal surface, one anterior and two posterior to the floor of the pharynx (asterisks). Head involution is blocked so the remnants of head structures remain on the outside. b, Suppression of abdominal features. Instead, mesothoracic denticles appear on the dorsal surface of the head (asterisks) and in trunk segments T1 to A8. Note the supernumerary A9 which bears prothoracic denticles (arrowhead) and the presence of chitinous plates in most body segments (arrows), which indicate transformation towards head structures (compare posterior end of *P9* embryo). c, Segments T2 to A7 show mesothoracic denticle bands, while the denticle band of A8 appears to be intermediate between normal T1 and T2. Note the presence of Keilin's organs (arrows) and black dots (arrowheads) in all trunk segments and the presence of chitinous plates posterior to A8 (asterisks). Dorsally, the segments T2 to A8 appear to be composed of anterior T2 and posterior T1, and a ninth abdominal segment is recognizable between the chitinous plates (asterisks) and the tuft. ap, Anal plate; as, anal sense organ; mx, maxillary sense organ; sp, posterior spiracles; tf, tuft. Other abbreviations as for Fig. 1.



expressed *Pc* group genes suggests a surprising complexity of the *trans*-regulatory system that controls the spatial expression of homoeotic genes. It is conceivable that such a complex system is better buffered against perturbation and thus better adapted in evolutionary terms than would be a spatial control by only one or a few *trans*-regulatory genes. If one were to view segment diversity as the result of evolutionary modification of a single repeated pattern¹², slight genetic changes in *trans*-regulatory genes controlling the spatial expression of homoeotic genes might gradually have built up the necessary genetic isolation between segments to ensure largely independent evolution of segment-specific features.

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Local degradation of fibronectin at sites of expression of the transforming gene product pp60^{src}

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Local degradation of extracellular fibronectin, a major extracellular adhesive protein, is believed to play an important part in the migration of cells through the extracellular matrix during tumour invasion, morphogenetic movement and trophoblast implantation¹⁻⁵. Fibronectin is lost from the cell surface after transformation with Rous sarcoma virus (RSV)⁶. By using fluorescent and radiolabelled probes covalently coupled to the surface of substrata, we have recently identified a proteolytic activity that is expressed in RSV-transformed cells and is involved in the local degradation of fibronectin at cell-substratum contact sites⁷. Here, we extend the relevance of these findings and gain some insight into the cellular functions of pp60^{src}, the transforming gene product of RSV. We show that newly expressed viral pp60^{src} is localized at the cytoplasmic surface of the cell membrane, corresponding to the cell contact sites where degradation of extracellular fibronectin occurs.

Localized fibronectin degradation was detected in cells cultured on fixed gelatin films coupled with rhodamine- or radio-labelled fibronectin in the presence of 10% fetal bovine serum. The appearance of black spots on the uniformly fluorescent surface of the gelatin film indicated sites of local fibronectin degradation. The resulting release of labelled fibronectin into the medium provided a measure of the cleavage of the polypep-

tides by the cells⁷. Because there was an induction of rosette contacts on RSV transformation of cells concomitant with a redistribution of α -actinin and vinculin to rosette contacts⁷⁻¹¹, we used monoclonal antibody N.353 directed against α -actinin for immunolabelling of the rosettes in the transformed cells in the above preparation, in order to determine whether the local degradation of fibronectin occurs at rosettes.

The system chosen for the study of cell transformation consisted of primary cultures of chicken embryo fibroblasts (CEF) infected with a temperature-sensitive mutant (ts68) of RSV¹². Infected cells exhibited the transformed phenotype when grown at the permissive temperature (37 °C) after a shift from the non-permissive temperature (41 °C). ts68-CEF grown at the permissive temperature for various times therefore allow a direct analysis of the transformed phenotype. Figure 1 shows that within 1 h after the temperature shift (Fig. 1a-c), a few black spots appeared on the rhodamine-fibronectin substrata (Fig. 1b) and these coincided with the immunoreactive α -actinin-rich rosette contacts of transformed cells (Fig. 1c). However, within 6 h after the shift (Fig. 1d-f), the cells became rounded, a morphological change that was accompanied by increasing numbers of degraded fibronectin spots (Fig. 1e) coinciding with α -actinin-rich rosette contacts (Fig. 1f). As shown previously in RSV-transformed cells⁷, the degraded spots appear at the rosettes (Fig. 1, open arrows), but never at the focal adhesions (Fig. 1, fluorescent streaks). In addition, the pattern of degraded spots is distinct from that of fibronectin displacement from non-coupled substrata exhibited by normal or transformed cells⁷. Furthermore, the initial release of radioactivity from the fibronectin substrata was detectable by 6 h after the temperature shift in ts68-CEF cultures (result not shown). The released material was characterized as peptides of relative molecular mass (M_r) < 30,000. Thus, localized degradation of extracellular fibronectin occurs at sites of rosette contacts of RSV-transformed cells.

Previous efforts to localize pp60^{src} all involved the use of polyclonal antisera^{9,10,13-15}. Because these antisera react with both cellular and newly expressed viral pp60^{src}, we examined

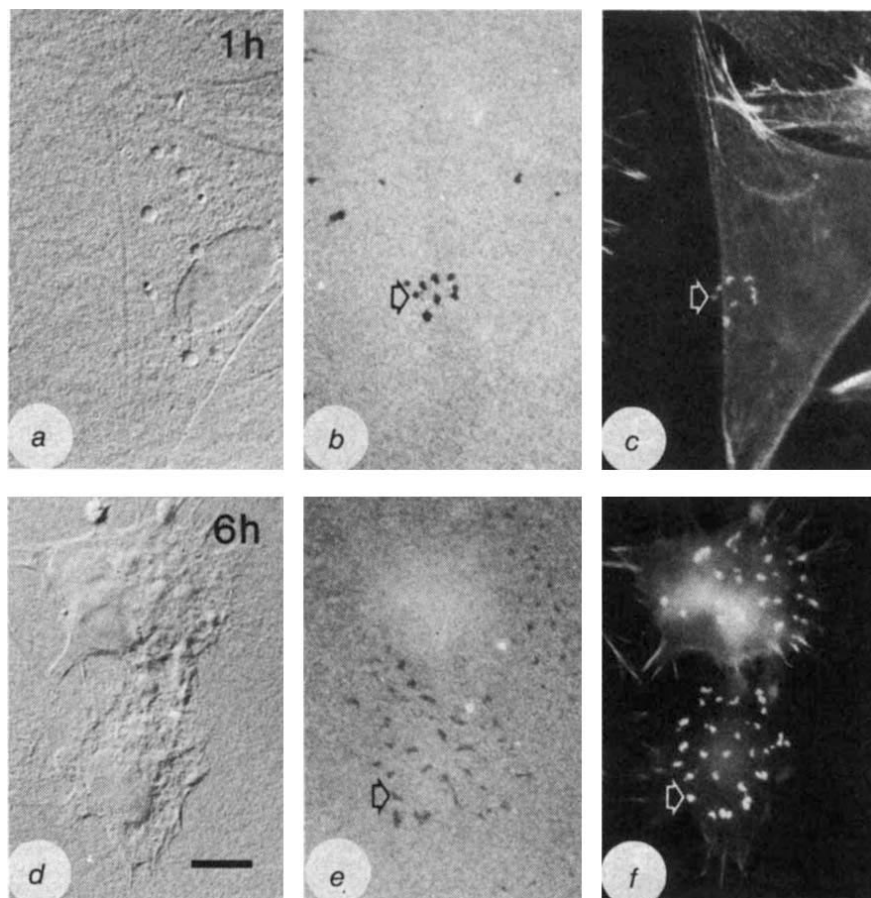
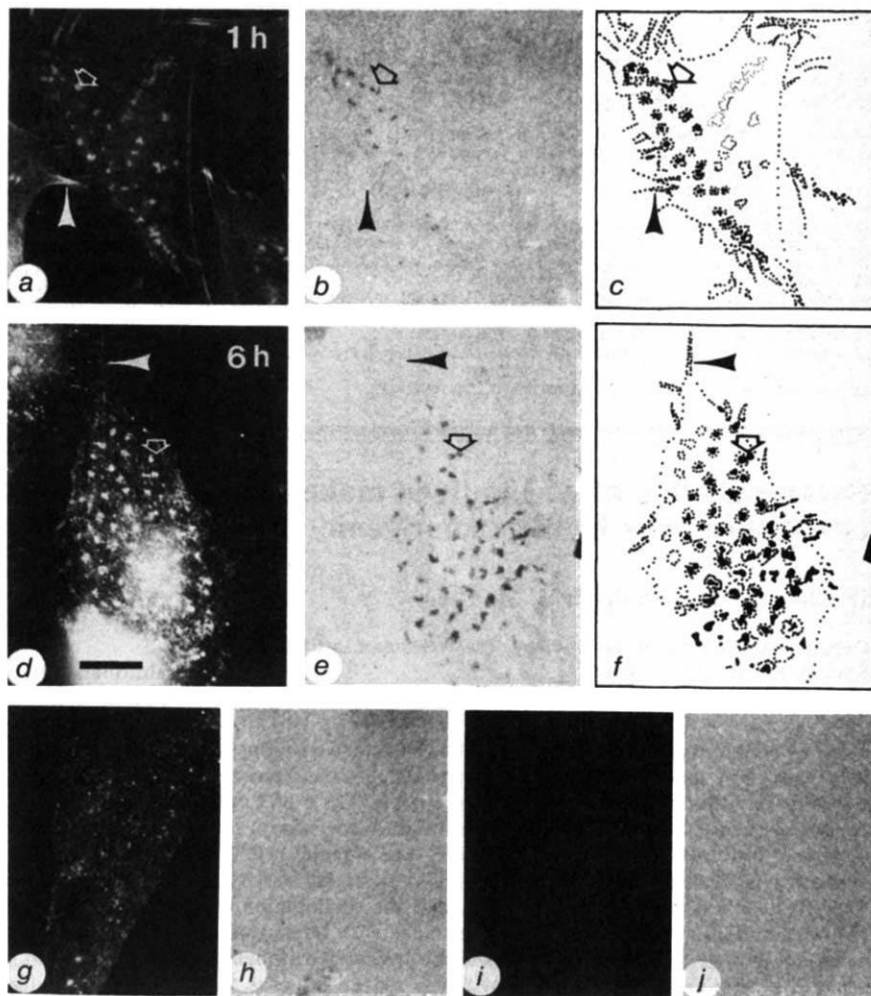


Fig. 1 Demonstration of the time course of expression of a transformation-associated protease that degrades fibronectin at rosette contact sites. ts68-infected CEF were grown at the permissive temperature (37 °C) for 1 h (a-c) and 6 h (d-f) after a temperature shift from 41 °C. a, d, Differential interference contrast images of the cells. b, e, Fluorescent images of rhodamine-labelled fibronectin substrata showing fields of the same cells as in a and d, respectively. Black spots (open arrowheads) represent areas where fibronectin has been locally degraded. c, f, Immunofluorescence images of the same cells as in a and d, respectively, using indirect monoclonal antibody labelling for intracellular α -actinin. α -Actinin is redistributed on transformation to rosette sites (open arrowheads) which coincide with the black spots in b and e.

Methods. The ts68-infected CEF were cultured in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum on rhodamine conjugates of fibronectin that were covalently linked to the surface of a fixed gelatin film¹⁷. The cultures were then shifted to the permissive temperature (37 °C) for various periods of time before fixation and permeabilizing with 0.5% Triton X-100 for antibody labelling. The fixed cells were labelled indirectly, first with mouse monoclonal antibody N.353 directed against α -actinin (Amersham Corp., Arlington Heights, Illinois), then with fluorescein conjugates of goat antibody to mouse IgG and IgM. Labelled cells were observed with a Zeiss Photomicroscope III equipped with epifluorescence and a Planapo 63/1.4 objective. Scale bar, 10 μ m.

Fig. 2 Coincidental localization of viral pp60^{src} and fibronectin degradation at sites of rosette contacts. Normal CEF (*i, j*) and ts68-infected cells were grown either at the non-permissive temperature (41 °C) (*g, h*), or at the permissive temperature (37 °C) for 1 h (*a-c*) and 6 h (*d-f*) after a temperature shift from 41 °C. The ts68 RSV-transformed cells were cultured on rhodamine-fibronectin substrata according to the procedure described in Fig. 1 legend, except that fixed cells were indirectly labelled with EB7 directed against an epitope of viral pp60^{src}. *a, d*, Immunofluorescence images of transformed cells labelled with EB7 for localization of intracellular viral pp60^{src}. Open arrowheads indicate rosette contacts that appear after cell transformation. White arrowheads point to focal adhesions, which are commonly found in both normal and transformed cells. *b, e*, The rhodamine-fibronectin substrata under the cells shown in *a* and *d*, respectively. Black spots represent sites of local fibronectin degradation, and are present at areas corresponding to rosette contacts (open arrowheads), but absent at focal adhesions (solid arrowheads) and areas free of cell contact. *c, f*, Overlay tracing of pp60^{src} localization in the cells of *a* and *d* (dotted outline) and of degraded fibronectin spots on substrata of the same fields (solid areas). *g, h*, Viral pp60^{src} labelling of ts68-CEF grown at 41 °C (*g*) and its underlying labelled fibronectin substrata (*h*). *i, j*, Control for viral pp60^{src} labelling in normal CEF (*i*) and the corresponding labelled fibronectin substrata (*j*). The same exposure time was used for photographing the viral pp60^{src} labellings in all preparations. Scale bar, 10 µm.



the intracellular localization of viral pp60^{src} by using monoclonal antibody EB7, which recognized an epitope in the amino-terminal region (residues 1-82) of the viral pp60^{src}, but did not efficiently recognize determinants on the cellular pp60^{src} homologue^{16,17}. To determine whether local removal of fibronectin also occurs at the sites of expression of viral pp60^{src} on RSV transformation, ts68-CEF were grown on rhodamine-fibronectin substrata at the non-permissive temperature (41 °C), shifted to 37 °C for various periods of time as described above, then immunolabelled with EB7 to compare the time-course correlation between viral pp60^{src} localization and the sites of fibronectin degradation. In normal CEF grown at 41 °C or 37 °C, we observed neither viral pp60^{src} labelling nor degraded spots (Fig. 2*i, j*). In ts68-CEF grown at 41 °C, viral pp60^{src} labelling was diffuse in the cytoplasm, but no local fibronectin degradation was detected (Fig. 2*g, h*). Within 1 h after the temperature shift to 37 °C, viral pp60^{src} was localized at both focal adhesions and rosette contacts (Fig. 2*a, c*), but mostly within rosettes at later times (Fig. 2*d, f*). Concomitantly, local fibronectin degradation was found mostly at rosettes, but not at focal adhesions (Fig. 2*b, c, e, f*). As shown in Fig. 1, however, there is not a perfect one-to-one correspondence between pp60^{src} and spots of fibronectin degradation, indicating that some of the rosettes either show little proteolytic activity or have become detached from the original sites. These results suggest a temporal and spatial relationship between rosette formation, local fibronectin degradation and viral pp60^{src} expression in RSV-transformed cells. It appears that viral pp60^{src} is produced in cells at the non-permissive temperature and becomes accumulated in functional sites at rosettes after the shift to the permissive temperature.

Functional expression of pp60^{src} is required for manifestation of the oncogenic events which are initiated on RSV infection^{12,18}.

Several studies have shown that viral pp60^{src} has the following characteristics: (1) it has an intrinsic tyrosine-specific phosphotransferase activity^{19,20}; (2) it is localized on the cytoplasmic face of the plasma membrane²¹; (3) its target proteins include the adhesion plaque component vinculin¹³; and (4) it has a cellular homologue²², which has been identified recently. The common localization for viral pp60^{src} and fibronectin degradation at rosette contacts demonstrated here suggests membrane-associated sites of action for both pp60^{src} and the extracellular protease. The pp60^{src} kinase may exert its primary effects on the plasma membrane at the rosettes through phosphorylation of integral membrane components²³ or of cytoplasmic vinculin^{9,13}. Such modification may directly or indirectly induce protease activity on the rosettes and thereby cause the local dissolution of fibronectin from the extracellular matrix. Such an effect could in turn have an important role in the local decrease in cell-substratum adhesion, in the rounding-up of the cell, and in the disorganization of the stress fibres that accompanies transformation of fibroblasts by RSV.

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Overproduction of p53 antigen makes established cells highly tumorigenic

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The p53 cellular tumour antigen, long known to be overproduced in a variety of neoplastically transformed cells^{1,2}, was recently shown to be directly involved in transformation. Thus, p53 can complement activated Ha-ras in transforming secondary rat embryo fibroblasts into grossly altered, tumorigenic cells^{3,4}. Moreover, p53 can also be shown to possess immortalizing activity⁵. Our previous results indicated, however, that the contribution of p53 to the transformation was not synonymous with immortalization, suggesting that the two activities of the protein are probably separable⁶. We demonstrate here that this is indeed the case, as overproduction of p53 in an established cell line, while not causing gross morphological changes, endows these cells with an overt tumorigenic potential. Furthermore, the tumorigenic efficiency of such cell lines may be correlated with the extent of p53 overproduction.

Co-transfection of rat embryo fibroblasts with a combination of activated Ha-ras and transcriptionally-potentiated p53 effectively generates morphologically transformed cells, capable of overgrowing the growth-arrested monolayer of normal cells^{3,4}. However, only a small fraction of such transformed cells are capable of continuous growth³. It was therefore proposed that

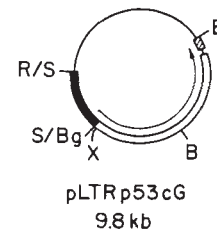
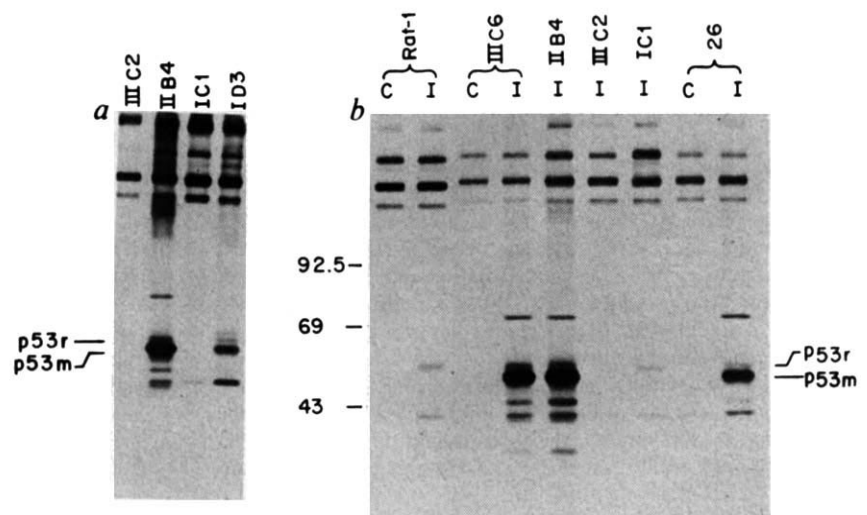


Fig. 1 Map of plasmid pLTRp53cG. To generate this construct, the DNA of plasmid pSVp53cG3 (ref. 12) was cleaved with *EcoRI* and *BglII* to remove simian virus 40 (SV40) sequences and then treated with the Klenow fragment of *Escherichia coli* DNA polymerase I to generate blunt ends. In parallel, a cloned proviral Harvey murine sarcoma virus (clone H-1; ref. 13), was cleaved with *SmaI*. The 1.3-kilobase fragment containing the retroviral 5'LTR (long terminal repeat) was isolated and introduced into the above blunt-ended vector. The resultant plasmid, pLTRp53cG, contains the LTR-derived enhancer, promoter and cap sites¹⁴, followed by a chimera of p53-specific DNA and genomic DNA¹². Solid bar, LTR sequences and adjacent 5' flanking cellular sequences; open bar, p53-specific sequences; thin line, pBR322 DNA; hatched bar, SV40 DNA. B, *BamHI*; Bg, *BglII*; R, *EcoRI*; S, *SmaI*; X, *XhoI*. Arrow indicates expected direction of transcription.

although nuclear oncogene products may have immortalizing potential, as was later in fact demonstrated for p53⁵, their ability to participate in neoplastic transformation may reflect an entirely different facet of their activity³. If this were the case, one might anticipate that certain types of established, non-transformed cells could be converted into more neoplastic ones by the mere overproduction of p53.

To test this assumption, we attempted to generate cell lines which over-produced the p53 antigen. Rat-1 cells⁶ were co-transfected with a mouse p53-specific expression vector together with a dominant selectable marker, ptkgpt (given by M. Shani). In the present study, we used a construct containing a chimera of p53 complementary DNA and genomic DNA (pLTRp53cG; Fig. 1). Rat rather than mouse cells were chosen because the p53 proteins of the two species can be easily differentiated with the aid of specific monoclonal antibodies, thus ruling out the possibility of endogenous rat p53 being nonspecifically induced by irrelevant parts of the transfecting plasmids. Mycophenolic-acid-resistant colonies were isolated⁷, expanded and analysed for the expression of mouse p53. Of 24 clones tested, most expressed little or no detectable murine p53. Only two lines displayed a very prominent overproduction of mouse p53, while two others made more moderate amounts of this protein. Figure 2 depicts the analysis of several such cell lines, using either a

Fig. 2 Detection of p53 in Rat-1-derived cell lines. Cells were grown in 60-mm dishes and labelled with 30 μ Ci ³⁵S-methionine per dish for 4 h. Proteins were extracted, immunoprecipitated and analysed by SDS-polyacrylamide gel electrophoresis as described previously⁸. Immunoprecipitation was with either monoclonal antibody RA3-2C2, specific for mouse p53¹⁵ (a), control serum (b, lanes labelled C) or monoclonal antibody pAb421, (L21)¹⁶, recognizing both mouse and rat p53¹⁵ (b, lanes I). Rat-1 is the parental cell line used for transfections; clone 26 is derived from rat embryo fibroblasts transformed by p53 plus Ha-ras³; all other cell lines were generated by co-transfection of Rat-1 cells with a p53-specific expression plasmid and ptkgpt. Each lane represents the polypeptides precipitated from 1.8×10^6 trichloroacetic acid-insoluble c.p.m. p53r and p53m denote the positions of rat and mouse p53, respectively. Numbers to the left of b refer to the relative molecular mass ($\times 10^{-3}$) of co-electrophoresed markers.



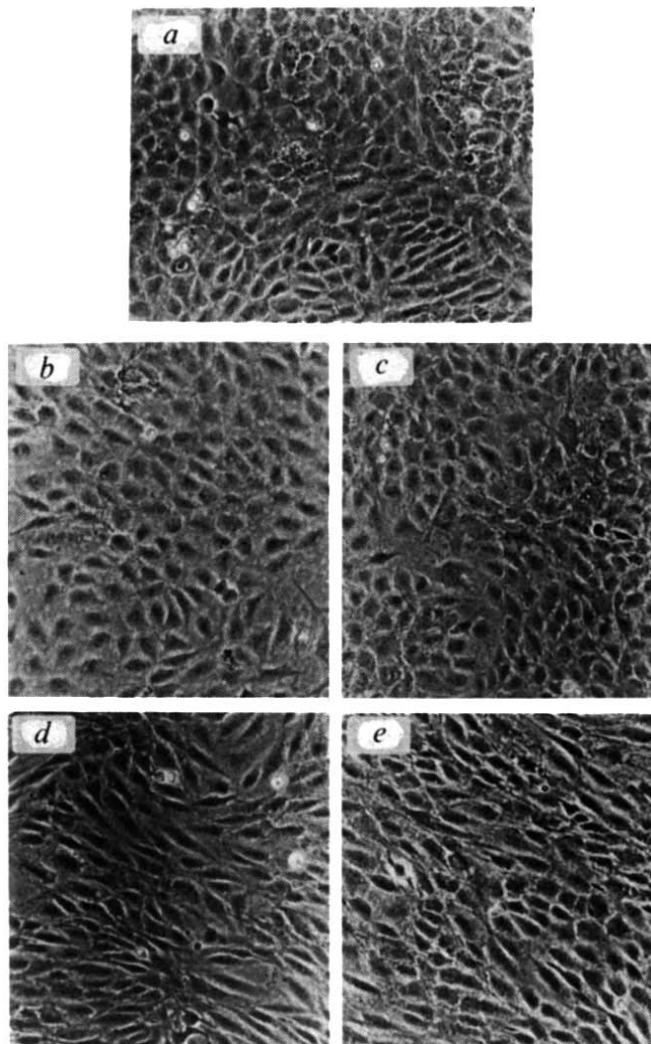


Fig. 3 Morphology of Rat-1-derived cells (phase-contrast photomicrographs). *a*, Parental Rat-1 cells; *b*, IB2; *c*, IIC6; *d*, IIB4; *e*, IIB4T, a cell line established from a tumour induced in a nude mouse by injection of IIB4 cells. $\times 83$.

mouse p53-specific antibody (Fig. 2) or one reactive with both the mouse and rat species (*b*). In two lines (IIB4 and IIC6), the production of mouse p53 well exceeds that of clone 26, a tumorigenic cell line derived from co-transfection of rat embryo fibroblasts with p53 plus Ha-ras (ref. 3 and D.E. *et al.* in preparation).

Unlike the case for p53 plus Ha-ras co-transformants, the p53-overproducing Rat-1 cells exhibited no dramatic morphological changes (Fig. 3). Some cell lines, such as IIB4, possessed a slightly altered shape while others, such as IIC6, were practically indistinguishable from the parental Rat-1.

Overproduction of p53 can sometimes be due to post-translational events^{8,9}. Hence, it was of interest to determine whether the augmented levels of the protein in these cell lines reflected, as expected, the presence of larger amounts of the corresponding messenger RNA. Total cytoplasmic RNA was prepared from several lines and analysed directly by electrophoresis on a denaturing gel and hybridization with a mouse p53 cDNA probe. Both IIB4 and IIC6, marked producers of the protein, contain conspicuous quantities of p53 mRNA (Fig. 4).

To test whether p53 overproduction could alter the biological properties of established cells, representative cell lines were injected into nude mice and monitored for the appearance of tumours. The results, summarized in Table 1, clearly demonstrate that both p53-overexpressing lines are highly tumorigenic, clone IIB4 being more efficient than IIC6. Since IIB4 appears

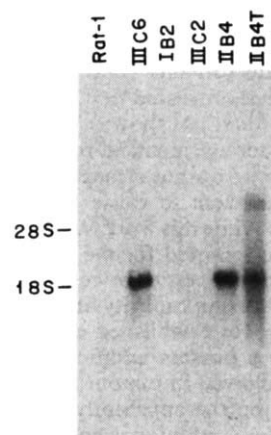


Fig. 4 Detection of p53-specific transcripts in various cell lines and in a tumour. Total cytoplasmic RNA was extracted from several cell lines (first five lanes) by the Nonidet-P40 method¹⁷. In parallel, total cellular RNA was prepared from a tumour induced in a nude mouse by the injection of IIB4 cells (lane IIB4T) using the guanidine thiocyanate procedure¹⁸. RNA (8 μ g) was electrophoresed through a formaldehyde agarose gel¹⁹, blotted onto nitrocellulose²⁰ and hybridized with the 950-base pair p53-specific cDNA insert of clone pp53-176 (ref. 21) as described previously²².

Table 1 Tumorigenicity of Rat-1-derived cell lines in nude mice

Cell line	Mouse p53	2 Weeks		4 Weeks		8 Weeks	
		Score	Size	Score	Size	Score	Size
Rat-1	—	0/4	—	0/4	—	1/4	4
IIC2	—	0/2	—	0/2	—	ND	
IIC6	++	0/5	—	4/5	3–5	5/5	3–12
IIB4	+++	8/11	4–7	11/11	8–20		

CD1 *nu/nu* mice were injected subcutaneously with 5×10^6 cells. The numbers and sizes of resultant tumours were monitored for 8 weeks after injection. Score values indicate the number of tumour-bearing mice over the total number of animals injected with the particular cell line. Also shown is the range of sizes (in mm) of the observed tumours. ND, not determined.

to make more p53 than IIC6, as determined by radiolabelling (Fig. 2), it is possible that the extent of p53-overexpression may determine the tumorigenic potency of these cells. It should be noted that a small tumour became visible after 8 weeks in one mouse injected with parental Rat-1 cells; this is in accord with the previously described ability of these cells to cause tumours with a long latency⁶. Thus, p53 may be markedly enhancing a latent tumorigenic potential of established cells rather than conferring such a potential *de novo*.

The rapid rate of tumour appearance following injection of IIB4 cells suggests strongly that their tumorigenicity is a property of the whole population rather than of a minor subset of different, fully transformed cells. Nevertheless, it was desirable to determine whether the cells comprising the tumour were not fundamentally different from those initially injected. To that end, we first determined the level of p53 mRNA in the tumour. The result (Fig. 4, lane IIB4T) clearly indicates that the tumour still makes very large quantities of p53 mRNA of the expected size; there is also a band of high relative molecular mass, not seen in tissue-cultured IIB4 cells. As the tumour RNA was extracted by a procedure which still retains the nuclear fraction, this higher band probably represents a precursor accumulating to high levels in the nuclei.

To further characterize such tumours, we excised one of them and placed it in tissue culture. The resultant cell line, IIB4T, had a morphology completely indistinguishable from that of the

parental IIB4 line (Fig. 3). This is in marked contrast to the results of a similar analysis on tumorigenic lines derived from p53 plus Ha-ras transformants (D.E. *et al.*, in preparation). Finally, protein analysis revealed that IIB4T possess the same level of p53 as IIB4 (data not shown), suggesting that the cells constituting the tumour are identical to those initially injected.

The data presented here argue strongly that the mere overproduction of p53 is sufficient to cause established cell lines to become tumorigenic. While this work was in progress, essentially similar findings were reported for the *myc* oncogene¹⁰, which also has several other properties in common with p53 (refs 3, 4, 11). As the immortalizing capacity of these nuclear oncogene products is irrelevant for established cell lines, one must conclude that both genes possess additional biological activities which are directly involved in tumour formation, while having no discernible effect on the apparently normal morphology of the cells. Whether these additional activities are the same for p53 and *myc* remains to be determined.

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Human N-myc gene contributes to neoplastic transformation of mammalian cells in culture

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Proto-oncogenes represent a group of eukaryotic genes whose activated forms are implicated in the development of cancer (for reviews, see refs 1-3). We have recently identified a human gene, *N-myc*, that is distantly related to the proto-oncogene *c-myc*⁴. *N-myc* is expressed at abnormally high levels consequent to amplification in numerous human neuroblastoma cell lines and metastatic neuroblastoma tumours^{5,6}. In addition, enhanced expression of *N-myc*, often a result of amplification, has been found in retinoblastoma cell lines and tumours (refs 5, 7 and M.S., unpublished data) and in cell lines derived from small-cell car-

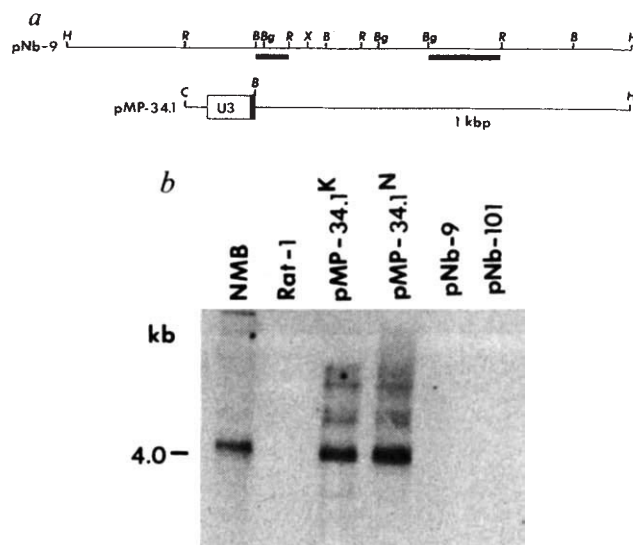


Fig. 1 Molecular clones of N-myc. *a*, Partial restriction endonuclease maps. The clones pNb-9 and pMP-34.1 were used for transfections. H, *Hind*III; R, *Eco*RI; B, *Bam*HI; Bg, *Bgl*II; X, *Xho*I. Heavy lines beneath the map of pNb-9 indicate the domains within which the 5' and 3' ends of N-myc must lie. Transcriptional orientation is from left to right. The U3 region in clone pMP-34.1 is derived from a M-MuLV LTR. The *Bam*HI site in the LTR used for attachment to N-myc is normally not present, but is the result of insertion of a polylinker in the natural *Kpn*I site within the R region of the Moloney murine leukaemia virus LTR. *b*, Expression of N-myc clones in Rat-1 cells. N-myc constructs were co-transfected together with a gene conferring resistance against the drug neomycin (molar ratio ~10:1) according to routine procedures¹⁰, and resistant cells were selected using the drug G418 (Gibco; 800 µg ml⁻¹ crude). Cells were then grown in mass culture, polyadenylated RNA was isolated from a cytoplasmic fraction, and 5 µg per lane was separated on a 1% agarose gel in the presence of 2.2 M formaldehyde. The RNA was transferred to nitrocellulose paper which was then probed with ³²P-labelled insert of plasmid clone pNb-1 (ref. 4). pNb-101 represents a cosmid clone containing a ~35 kb insert encompassing N-myc. NMB represents Kelly, a human neuroblastoma cell line.

cinomas of the lung⁸. Here, we show that enhanced expression of N-myc subsequent to co-transfections of an N-myc expression vector and the mutant c-Ha-ras-1(EJ) (from the human bladder carcinoma cell line EJ) is a factor in tumorigenic conversion of secondary rat embryo cells. The transformed cells elicit tumours in athymic mice and isogenic rats. The ability of N-myc to contribute to neoplastic transformation of cultured mammalian cells raises the possibility that enhanced expression consequent to amplification of N-myc may be a factor in the aetiology of human neuroblastoma.

To design a suitable N-myc expression vector, we have generated a preliminary definition of the boundaries of N-myc in human DNA. We found that N-myc is localized within an ~16.0-kilobase (kb) *Hind*III fragment (Fig. 1a; clone pNb-9) (M.S. *et al.*, manuscript in preparation). The 5' end of N-myc appears to reside within a 1.0-kb *Bam*HI/*Eco*RI fragment; the 3' end maps within a 2.0-kb *Bgl*II/*Eco*RI fragment in close proximity to the *Eco*RI restriction endonuclease site. A DNA fragment containing the U3 region of a long terminal repeat (LTR; clone pMP-1) of Moloney murine leukaemia virus (M-MuLV) was covalently linked to N-myc derived from the Kelly neuroblastoma cell line and from a normal individual, to generate clones pMP-34.1^K and pMP-34.1^N, respectively. Rat-1 cells, into which either one of these clones was introduced by transfection of DNA co-precipitated with calcium phosphate⁹, produced high levels of an ~4.0-kb N-myc transcript, as do human neuroblastoma cells containing amplified N-myc (Fig. 1b). N-myc messenger RNA was not detected in Rat-1 cells into which

Table 1 Transformation of rat embryo cells

Gene transfected	Clone*	No. of foci†
LTR/N- <i>myc</i> (Kelly) + c-Ha- <i>ras</i> -1 (EJ)	pMP-34.1 ^K + pEJ(6.6)	30-40
LTR/N- <i>myc</i> (normal) + c-Ha- <i>ras</i> -1 (EJ)	pMP-34.1 ^N + pEJ(6.6)	30-40
LTR/N- <i>myc</i> (Kelly; mutant) + c-Ha- <i>ras</i> -1(EJ)	pMP-34.1 ^K ΔXho + pEJ(6.6)	0
N- <i>myc</i> (Kelly) + c-Ha- <i>ras</i> -1 (EJ)	pNb-9 ^K + pEJ(6.6)	0
N- <i>myc</i> (normal) + c-Ha- <i>ras</i> -1 (EJ)	pNb-9 ^N + pEJ(6.6)	0
N- <i>myc</i> (Kelly) + LTR	pNb-9 ^K + pMP-1	0
N- <i>myc</i> (normal) + LTR	pNb-9 ^N + pMP-1	0
c-Ha- <i>ras</i> -1 (EJ) + LTR	pEJ(6.6) + pMP-1	0
LTR/N- <i>myc</i> (Kelly)	pMP 34.1 ^K	0
LTR/N- <i>myc</i> (normal)	pMP 34.1 ^N	0
c-Ha- <i>ras</i> -1	pEJ(6.6)	0
LTR	pMP-1	0

Primary cultures of rat embryo cells (REC) were prepared from 12-14-day-old Fisher rat embryos. After 2-4 days of growth in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum (FCS; Hyclone) and antibiotics, the cells were seeded at a density of 1×10^6 cells per 100-mm dish. Transfections were performed using 75 µg of carrier DNA from salmon sperm plus roughly equimolar amounts of DNA of the various constructs (6.6 µg pEJ (6.6); 13 µg pMP-34.1; 16 µg pNb-9; 1 µg pMP-1) for two dishes which at the time of transfection contained 4×10^6 cells. About 24 h after transfection, the cells were split at a ratio of 1:3, re-fed at 3-day intervals with DMEM plus 5% and inspected for focus formation.

* For clones pMP-34.1 and pNb-9 see Fig. 1a. The mutated LTR/N-*myc* was generated by linearizing clone pMP-34.1^K at the single *Xho*I restriction endonuclease site (Fig. 1a), recutting the protruding single-stranded ends, followed by religation, thus creating a 4-base pair deletion. For clone pEJ (6.6) see ref. 11. Clone pMP-1 contains the U3 region of M-MuLV long terminal repeat (LTR).

† Based on three different experiments each involving $\sim 4 \times 10^6$ cells at the time of transfection.

clones pNb-9^K and pNb-9^N or cosmid clone pNb-101 (map not shown) had been introduced. We observed no obvious morphological differences between untransfected Rat-1 cells and those expressing high levels of human N-*myc* mRNA.

Previous studies¹⁰ had shown that second-passage rat embryo cells undergo neoplastic transformation when either a viral *myc* gene or a truncated c-*myc* from a murine plasmacytoma cell line, attached to a transcriptional enhancer, is co-transfected with c-Ha-*ras*-1(EJ). Co-transfection of rat embryo cells with the N-*myc* expression vectors pMP-34.1^K and pMP-34.1^N (Table 1) and c-Ha-*ras*(clone pEJ6.6; ref. 11) resulted in formation of foci between 7 and 10 days after transfection at a frequency of ~ 30 -40 foci per 4×10^6 cells. There was no obvious difference in the efficiency of N-*myc* genes derived from neuroblastoma line Kelly and of those from the normal individual. We did not obtain focus formation with any one of the control clones (Table 1). In particular, focus formation did not occur when we used an N-*myc* expression vector containing a frameshift mutation within N-*myc*, showing that enhanced expression of the product encoded by N-*myc* is required for neoplastic transformation.

Closer inspection revealed two morphologically different types of foci (Fig. 2): type I consisted of disorganized, loosely arranged, small, round to ovoid, refractile cells (Fig. 2a). The cells never reached high density within the focus, but floated off the bottom of the plate. Cells of individual foci grew in a three-dimensional pattern to high density (up to 2×10^8 cells per 100-mm dish (Fig. 2d,f). Foci of type II consisted of elongated fibroblastic cells which grew in an organized pattern tightly attached to the surface of the plate (Fig. 2c). Individual foci grew into a flat monolayer (density up to 2×10^7 cells per 100-mm dish; Fig. 2e, g). The fraction of type II foci amounted to ~ 10 -20% of the total number of foci. The emergence of two different types of foci following co-transfection of N-*myc* and c-Ha-*ras*-1(EJ) cannot be fully explained at present. These different morphologies have not been reported for experiments in which c-*myc* or v-*myc* were used¹⁰. It is possible that different levels of N-*myc* and/or Ha-*ras* mRNA account for the different phenotypes of neoplastically transformed cells. Another possibility is that cells of different embryonic lineages present in the second-passage embryo cell cultures assume different phenotypes following uptake of N-*myc* and c-Ha-*ras*-1(EJ). Cells of all foci could readily be established as permanent lines, and all cells of individual foci appeared to start growing without delay.

Approximately 10^4 cells of foci of type I, when injected subcutaneously or intraperitoneally into athymic mice and

isogenic rats, formed tumours that caused death within 2-3 weeks. The results were not affected by irradiation of the animals before injection of the cells. Cells of type II foci did not yield tumours even when 5×10^7 cells were injected into X-ray-irradiated animals.

Cells of eight foci selected from two independent transfection experiments all contained 15-30 copies of N-*myc* and 10-20 copies of c-Ha-*ras*-1 (data not shown). Cells of all foci contained a major N-*myc* mRNA of 4.0 kb. The concentration of N-*myc* mRNA varied considerably among cells derived from different foci but did not correlate with the amount of N-*myc* DNA. Cells of type I foci generally contained high concentrations of N-*myc* mRNA (Fig. 3, lines VI-a, -d, -e and -k; VII-b, -c), as much as

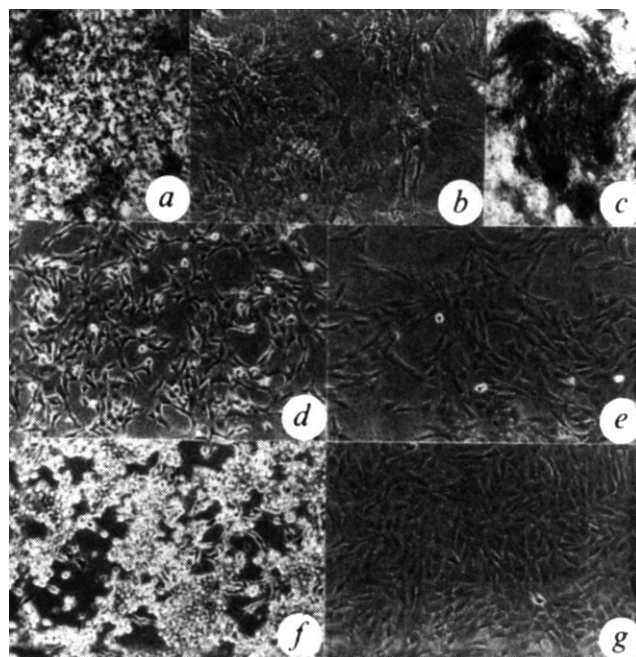


Fig. 2 Photomicrographs of rat embryo cells showing normal cells (b), and the morphology of foci of type I (a) and type II (c), resulting from transformation by LTR/N-*myc* and c-Ha-*ras*-1 (EJ). d, f, Transformed cells of focus type I (cell line VI-d) at low and high density, respectively. e, g, Cells of focus type II (line VI-j) at low and high density, respectively.

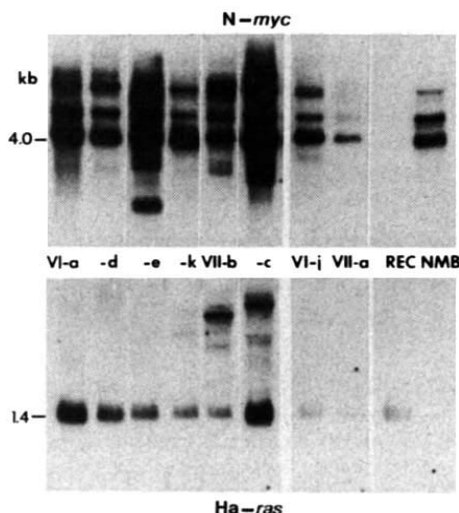


Fig. 3 Expression of *N-myc* and *c-Ha-ras-1* (EJ) in rat embryo cells transformed by LTR/*N-myc* and *c-Ha-ras-1* (EJ). Individual foci of transformed cells were cloned and grown into mass culture. Total polyadenylated RNA was isolated and analysed by agarose gel electrophoresis. VI-a, -d, -e and -k, and VII-c are cells derived from type I foci; VI-j and VII-a are cells from type II foci. REC, rat embryo cells; NMB, human neuroblastoma cell line. Each lane contains ~5 µg of RNA. The probe for *N-myc* was the *Eco*RI/*Bam*HI insert of pNb-1; for *ras*, the *Eco*RI insert of clone BS9 was used¹⁹.

10 times the level found in the neuroblastoma line NMB. Cells of type II foci generally contained lower levels of *N-myc* mRNA (lines VI-j, VII-a) that resembled the levels in neuroblastoma cell line NMB, although the level was in some instances only slightly lower than in type I cells (compare VI-d or -k with VI-j). There was apparently no absolute correlation, however, between the transformed phenotype of the cell and the level of *N-myc* mRNA: the level in the highly tumorigenic line VI-d was only slightly higher than that in the non-tumorigenic line VI-j.

In contrast, there seemed to be a correlation between the level of *Ha-ras* mRNA and the cellular morphology (Fig. 3). Levels of mRNA in cells of type I foci were three to five times higher than in rat embryo cells; in cells of type II foci, the level was not detectably higher than in rat embryo cells. The implications of this correlation are not clear. One possibility being tested is whether *N-myc* is capable of immortalizing rat embryo cells in culture.

N-myc, *c-myc* and *v-myc* apparently contribute in similar ways to transformation of early-passage embryo cells. The fact that at least one neuroblastoma cell line carries amplified *c-myc* instead of amplified *N-myc*¹² and, conversely, that several lines of cells from small-cell carcinoma of the lung have amplified *N-myc* instead of amplified *c-myc*⁷, indicates that there may also be a functional relation between *N-myc* and *c-myc* in the aetiology of naturally occurring tumours. It is not clear, however, that the similarities among the nucleotide sequences of *N-myc*, *c-myc* and *v-myc* are responsible for the apparent functional relationships. The function of *myc* genes in neoplastic transformation of cultured cells apparently can also be assumed by genes whose protein products are related to that of *myc* in regions other than the *N-myc/c-myc* similarity¹³, such as adenovirus early region 1A (ref. 14), or by genes whose relation to *myc* is not clear at present, such as those encoding the polyoma large-T antigen¹⁴ or the cellular tumour antigen, p53¹⁵⁻¹⁷.

We have found previously that enhanced expression and amplification of *N-myc* are correlated with stage III and IV neuroblastomas, characterized by poor prognosis⁸. It is not clear how the biological activity of *N-myc* in cultured cells relates to amplification of the gene in advanced neuroblastomas. The assessment of the role of *N-myc* in human neuroblastoma is complicated by the fact that classification of a specific stage of neuroblastoma is mainly based on the clinical picture at the

time of diagnosis rather than on cellular parameters; moreover, it is not known whether tumours of higher stages develop from lower-stage tumours. The correlation of *N-myc* amplification with stage III and IV neuroblastomas may indicate, therefore, that neuroblastomas comprise several classes of tumours, only one of which is characterized by *N-myc* amplification. We suggest this because primitive neuroectodermal tumours are characteristically difficult to distinguish from one another¹⁸; the heterogeneity among neuroblastomas now suggested by amplification of *N-myc* in only some tumours could therefore have gone undetected by more conventional criteria.

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Note added in proof: In a separate set of experiments we have now shown that *N-myc*, when co-transfected into rat embryo cells together with a gene conferring resistance against neomycin, is capable of both inducing an increased number of neomycin-resistant cells and establishing, for more than 120 cell divisions so far, host cells with morphology similar to that of cells derived from type II foci. These cells have a higher serum requirement than those derived from type I foci (details will be published elsewhere).

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The class I major histocompatibility antigen gene activated in a line of SV40-transformed mouse cells is *H-2D^d*, not *Qa/Tla*

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We have previously described several complementary DNA clones isolated because they correspond to messenger RNAs present at higher levels in the simian virus 40 (SV40)-transformed BALB/c 3T3 cell line SV3T3 Cl38 than in the normal, parental BALB/c 3T3 line¹. One of these clones, pAG64, hybridizes to RNAs which, while present in BALB/c 3T3 cells, are 10-20-fold more abundant in SV3T3 Cl38 and are found at high levels in a wide variety of transformed cell lines^{1,2}. Nucleotide sequence analysis showed that pAG64 encodes a class I antigen of the major histocompatibility

complex². To ascertain the identity of pAG64, we compared its sequence with the available sequences of *d* haplotype class I antigen genes [*K* locus³, *L* locus⁴, *D* locus⁵ and the *Qa* gene defined by genomic clone 27.1 (ref. 6)] and found that it showed multiple clustered differences from each of these sequences². We therefore concluded that it was not derived from the *H-2K^d*, *H-2L^d* or *H-2D^d* genes and thus must correspond to one of the other class I antigen genes, namely those of the *Qa/Tla* complex², although it was clearly not the *Qa* gene defined by the genomic clone 27.1. We now report subsequent findings which indicate that pAG64 in fact corresponds to the *H-2D^d* gene and not to a *Qa/Tla* gene.

We synthesized an oligonucleotide (nucleotides 716-733 in the sequence of pAG64) which should distinguish pAG64 from the other sequenced class I antigen genes of the *d* haplotype and used it to screen the BALB/c cosmid library of Steinmetz *et al.*⁷. We found that the oligonucleotide hybridized to the cosmid c18.1, which carries the *H-2D^d* gene, and to the cosmid 65.1 which carries two genes, 3 and 4 of cluster 1, from the *Qa2,3* region. Hybridization to the *H-2D^d* gene was unexpected in view of the sequence of this gene published by Margulies *et al.*⁵. Sher *et al.* have now reported⁸ the sequence of the cosmid c49.2, which carries the coding sequence of the *H-2D^d* gene. It is essentially identical to that of pAG64. Our conclusion that pAG64 did not correspond to *H-2D^d* was based on the sequence of the genomic clone Ch4a-D^d (ref. 5). Margulies *et al.* now report that the published sequence of this clone is incorrect⁹. They have re-determined the sequence of part of this clone and find that of 821 nucleotides sequenced, 821 are identical to the sequence of Sher *et al.* and that in the region of overlap, 299 nucleotides out of 300 are the same as pAG64. We therefore conclude that pAG64 corresponds to the *H-2D^d* gene and not to a *Qa/Tla* gene.

The other reason that led us to assign pAG64 to *Qa/Tla* was our inability to detect homologous transcripts in various normal mouse tissues which express H-2 but not *Qa* and *Tla* antigens. We now know that this was an artefact of our mRNA isolation procedure. All of the samples used in our previous publications were prepared by lysing the cells in a buffer containing Nonidet P-40, separating nuclei from cytoplasm and extracting RNA from the cytoplasmic fraction¹⁰. However, when we extract total RNA by the guanidine isothiocyanate procedure¹¹, we observe, as expected, appreciable levels of RNA homologous to pAG64 in various mouse tissues. We had always reprobed our blots with a cDNA clone for the mitochondrial transcript encoding cytochrome oxidase subunit 1 and had observed a clear band of the expected size and intensity. Therefore, it seems that the use of mitochondrial probes does not represent an adequate control. We stress that, irrespective of the method of RNA isolation, we observe much higher levels of class I mRNA homologous to pAG64 in SV40-transformed cells than in their normal parents. Similar results have since been reported by others for other virus-transformed and virus-infected cells^{12,13}.

We conclude that pAG64 is a cDNA clone corresponding to the *H-2D^d* gene and that high levels of this RNA are present in the SV40-transformed cell line SV3T3 Cl38. Whether the abundant class I antigen transcripts detected in other transformed cell lines are all derived from the *H-2D* gene is not known. Although our discussion of the possible roles of *Qa/Tla* antigens in normal and transformed cells² should be disregarded, the induction of class I antigen gene expression in transformed cells is clearly of interest and we are presently analysing the mechanisms involved¹⁴.

We regret the confusion that the mis-assignment of this clone has caused.

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An enhancer-like sequence within the *Xenopus* U2 gene promoter facilitates the formation of stable transcription complexes

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Enhancers are eukaryotic promoter elements that increase transcriptional efficiency in a manner relatively independent of their position and orientation with respect to a nearby gene¹⁻³. There is growing evidence that enhancer action is mediated by *trans-acting* factors⁴⁻⁸, but the mode of action of these factors is not yet known. We report here on the *Xenopus* U2 gene promoter, which contains two sequence elements. The distal sequence element increases promoter activity 20-fold by facilitating the formation of stable transcription complexes. A synthetic 14-base-pair (bp) oligonucleotide corresponding to part of the distal sequence element, which shows homology to an immunoglobulin gene promoter element and to both the simian virus 40 (SV40) and the immunoglobulin heavy-chain gene enhancers, stimulates transcription in an orientation-independent manner.

The promoter of the *Xenopus* U2 snRNA gene¹⁰, in common with other vertebrate U snRNA genes¹¹⁻¹³, has no sequences homologous to the standard elements (TATA box, CCAAT box) found in the 5' control regions of most RNA polymerase II transcribed genes (reviewed in ref. 14). Pioneering studies on the human U1 gene promoter^{12,15} have shown that sequences essential for transcription of this gene in *Xenopus* oocytes lie between 203 and 230 bp upstream of the cap site. Recent work has shown that efficient transcription of a human U2 gene requires sequences located between 258 and 198 base pairs upstream of the cap site¹⁶, although 62 base pairs of 5' flanking sequence are sufficient to accurately direct transcription initiation in *Xenopus* oocytes⁹.

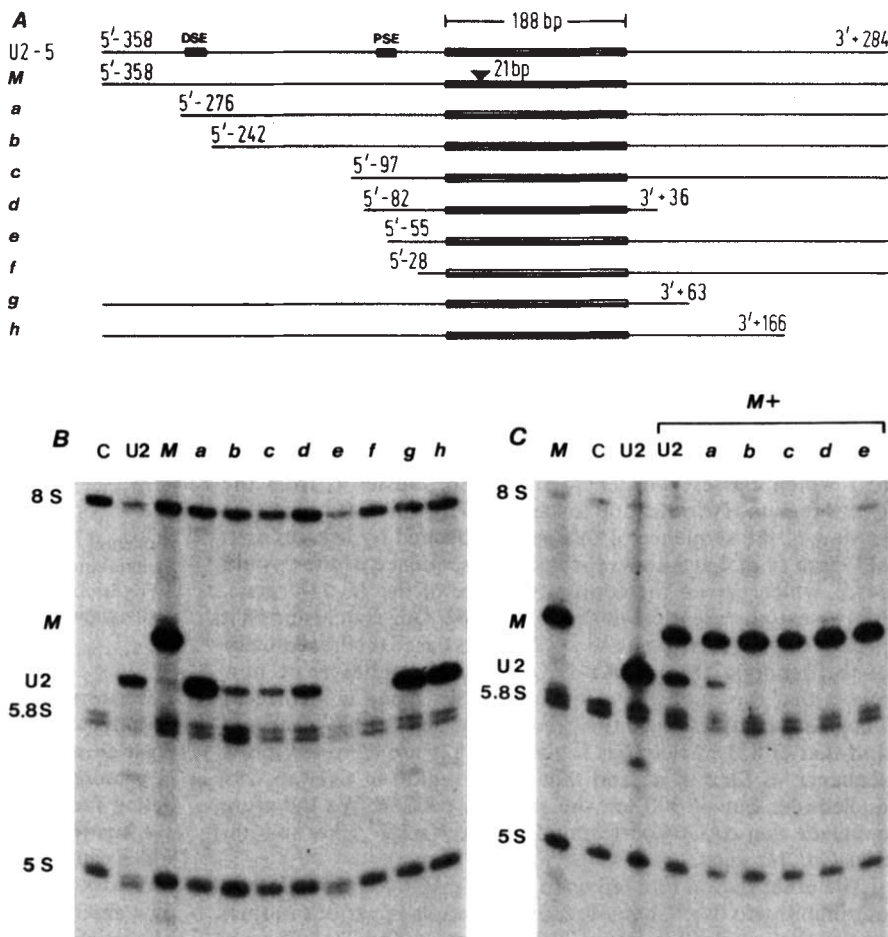
We have analysed the *Xenopus* U2 gene promoter by constructing a series of deletions removing parts of the 5' and 3' flanking sequences and assaying the transcription of the deletion mutants following microinjection into the nuclei of *Xenopus* oocytes. The extent of the deletions is shown in Fig. 1A. The gene indicated as *M* is a maxigene created by inserting 21 bp into the *Sau3AI* site between positions 28 and 32 of the U2 coding sequence¹⁰. The transcripts arising from mutants *a-h* are shown in Fig. 1B. Three conclusions can be drawn from the results shown in Fig. 1B. First, deletion of sequences between -82 and -55 (including the proximal element) eliminates U2 transcription (Fig. 1B, compare lanes *d*, *e*). Second, removal of sequences between -276 and -242 reduces the efficiency of transcription 10-20 fold (lanes *a*, *b*). No further reduction in transcriptional efficiency is seen on deletion of the sequences between -242 and -82 (lanes *b*, *c*, *d*). Third, deletion of sequences 3' to the U2 coding sequence up to 36 bp from the end of the coding sequence has no apparent effect on transcription (lanes *g*, *h*, *d*).

We next co-injected equal quantities of deletion mutant and

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Fig. 1 Transcriptional activity of mutant U2 genes with progressive deletions of flanking sequences. **A**, Diagrammatic representation of the extent of the various deletions. U2-5 was recloned from pXIU2-5 (ref. 10) into M13mp8 (ref. 28) and in this paper is referred to as wild-type U2. The 188-bp coding sequence is indicated as well as the length of flanking 5' and 3' sequence remaining. DSE and PSE are the distal and proximal sequence elements, and are described in the text. **M** is a maxigene made by the insertion of 21 bp into the U2 coding sequence. All the deletions are cloned in the same orientation in M13mp8 (ref. 28). **B**, Transcription of the U2 mutants after microinjection into *Xenopus* oocytes. Lane C, control oocyte, injected only with [α^{32} P]GTP. Lanes U2-h, oocytes injected with either U2 or one of the mutant U2 genes in Fig. 1A. The lanes are lettered corresponding to the mutant injected. U2 and maxigene transcripts are indicated, as are the endogenous 8S¹⁰, 5.8S, and 5S rRNAs. **C**, Transcription of U2 mutants after coinjection with the U2 maxigene. Lane C, control oocyte. Lanes U2 and M, oocytes injected with only U2 or maxigene DNA. Lanes M+U2 to M+e, oocytes coinjected with an equal amount of maxigene DNA and either U2 or mutant a to e DNA.

Methods. The construction of mutants a-c and e-h is described elsewhere²⁹. Mutant d is a *Fnu*4HI restriction fragment of pXIU2-5¹⁰, repaired with T4 DNA polymerase and cloned into *Sma*I digested M13mp8 (ref. 28). The maxigene was constructed by sub-cloning the two *Sau*3AI fragments of pXIU2-5 separately into the *Bam*HI site of M13mp8, excising them with either *Eco*RI-*Hinc*II or *Hind*III-*Sma*I double digests, and ligating them back into *Eco*RI-*Hind*III digested M13mp8 in their original configuration with the insertion of an *Xho*I linker between the *Sma*I and *Hinc*II ends. Microinjection and transcript analysis were performed exactly as in ref. 10. Relative transcription levels were estimated by scintillation counting of excised bands.



maxigene DNA to have an internal standard when comparing the transcriptional efficiency of various mutants. The results of this experiment are shown in Fig. 1C. The wild-type U2 and the maxigene are roughly equally transcribed on coinjection (Fig. 1C, lane M+U2). Mutant a, deleted from -358 to -276, which shows wild-type activity when injected alone (Fig. 1B, lane a), can still compete, although with a reduced efficiency compared to wild-type U2, when coinjected with maxigene (lane M+a). Mutants b, c and d, all of which are transcribed although at a reduced level when injected singly (Fig. 1B, lanes b-d) do not produce transcripts detectable above the level of endogenous U2 when co-injected with maxigene (Fig. 1C, compare lane M with lanes M+b, M+c, M+d).

The *Xenopus* U2 genes are tandemly repeated¹⁰. A construct which includes all the sequences up to -652, that is, to the prior coding sequence, behaves identically to the U2 clone with 358 base pairs of 5' flanking sequence in both single injection and coinjection experiments (data not shown), indicating that all the 5' flanking promoter elements necessary for full transcriptional activity in oocytes lie between -358 and -1. These results suggest that there must be a factor in oocyte nuclei whose concentration is rate-limiting for U2 gene transcription in these experiments. Sequences upstream of -242 are necessary for efficient association with this factor, such that when a gene with these sequences and a mutant in which they have been deleted are placed in direct competition, only the gene with the distal sequence element will be transcribed. In the absence of competition the factor can weakly associate with mutant genes lacking the distal sequence element, resulting in a lower level of transcription.

Mutant genes having only the proximal sequence element are thus weakly transcribed. We have made promoter constructs in which the proximal sequence has been deleted but in which the distal sequence element remains. These do not give rise to transcripts (data not shown) showing that the proximal sequence element is essential for transcription.

A characteristic of transcription complexes formed on genes transcribed by RNA polymerases III^{17,18} and I¹⁹ is that once formed, they are stable. Transcription factors remain closely associated with their DNA binding sites during many rounds of transcription. The competition experiments described above suggest that the distal sequence element is necessary for the association of a transcription factor. To test whether this association is also stable (through many rounds of transcription), competition experiments were performed in which two U2 constructs were microinjected consecutively rather than simultaneously. The first four lanes of Fig. 2 show that prior injection of vector DNA has no effect on the transcription of subsequently injected U2, maxigene, or mutant d DNA. This means that factors required for transcription do not associate stably with vector DNA. When U2 and maxigene DNA are coinjected, both are equally transcribed (Fig. 1C, lane M+U2). When they are consecutively injected, however, only the DNA injected first is efficiently transcribed (Fig. 2, lanes M+U2 and U2+M). This result suggests that transcription factors, at least one of which is limiting in concentration in these experiments, associate with the first injected DNA during the 3-h incubation, and that these complexes do not dissociate significantly during the following 20 h, that is, during many rounds of transcription. We estimate from Northern hybridizations that at least 10 transcripts per

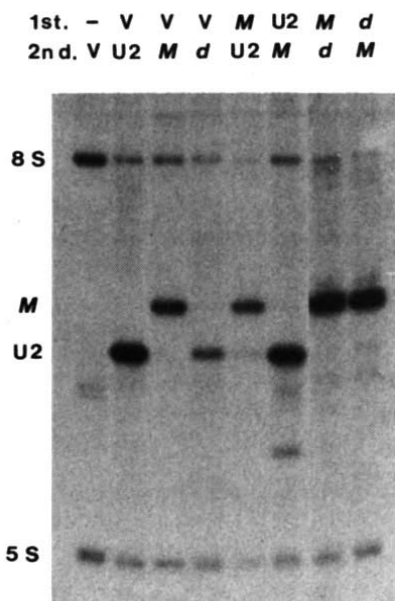


Fig. 2 Sequential injection of wild-type and mutant U2 genes. Oocytes were microinjected into the nucleus with one DNA, incubated for 3 h, microinjected with a second DNA, incubated for a further 3 h, then microinjected into the cytoplasm with [α^{32}]GTP. Twenty hours later RNA was extracted and analysed from single oocytes. The DNAs injected first and second are indicated. V, vector (M13mp8) DNA; U2, U2 DNA; M, maxigene DNA; d, mutant d DNA. Although only a single oocyte for each combination is shown, between 10 and 30 single oocytes were analysed in each case. The oocytes shown are representative of the most frequent (at least 70%) result obtained with each combination of injections. Some oocytes do not conform to the most frequent result, due to inaccurate microinjection such that some or all of one of the DNAs injected was not deposited in the nucleus, and thus was not transcribed. The main result, that genes lacking the distal sequence element cannot form stable transcription complexes, was obtained in three different experiments involving analysis of 30 individual oocytes.

injected gene accumulate during a 20-h incubation (data not shown). As the proportion of the injected genes assembled into active transcription complexes is unknown, the actual figure of transcripts per active gene could be significantly higher.

To determine the sequence requirements for this stable binding, we injected consecutively maxigene and mutant DNAs. The results for mutant d (Fig. 2) and mutant b (not shown), both of which lack the distal sequence element, were identical. Independent of whether the mutant DNA was injected before or after the maxigene, only the maxigene was efficiently transcribed (Fig. 2, lanes M + d and d + M). This shows that sequences >242 bases upstream of the cap site, likely to be identical to the distal sequence element identified in the previous section, are required for the formation of stable transcription complexes.

A sequence comparison of the region between positions -242 and -358 with other sequenced U snRNA genes revealed that homologous sequences are found in a *Xenopus* U1 gene¹² and a human U1 gene^{12,15} (Table 1). The human homology is part of the stretch between -203 and -230 previously shown to be necessary for transcription of this gene in *Xenopus* oocytes¹⁵. The *Xenopus* U1.3 gene can compete efficiently with the U2 gene on coinjection. Removal of sequences upstream of -216 from XIU1.3, including the homologous region shown in Table 1, results in the loss of this competitive ability (data not shown). A related sequence occurs in the promoter of immunoglobulin heavy-chain genes^{20,21} (Table 1), and in the promoters of immunoglobulin light-chain genes in the opposite orientation^{20,21}. A strongly homologous sequence is also present in the SV40 enhancer^{21,22} and, in the opposite orientation, in the

Table 1 A conserved region of the distal sequence element

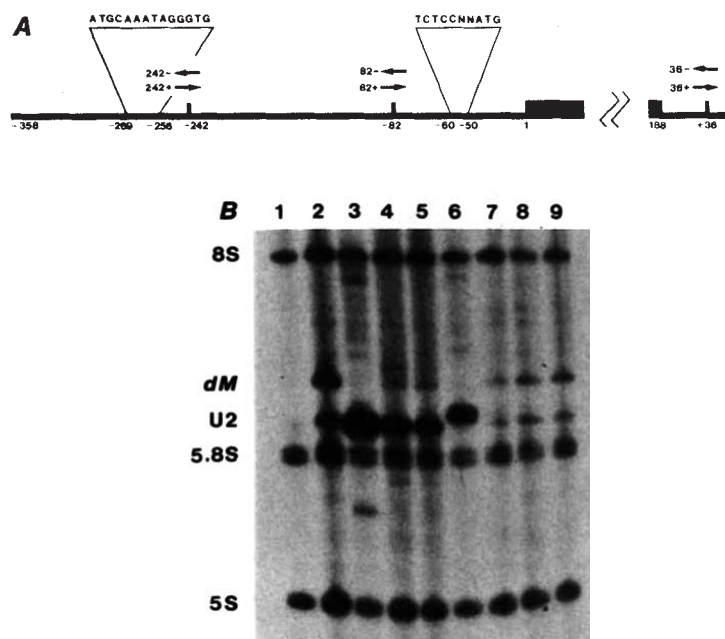
XIU2-5	AAGAGGCGGGGCTATGCAAAT-AGGGTG	-256
XIU1.5	TTGAAAAT-AGTCTG	-261
H.U1	ATGTAGATGAGGCAG	-205
Mammal Ig heavy-chain promoter	ATGCAAATNA	-60
SV40 enhancer	ATGCAAAGCA	
Mammal Ig heavy-chain enhancer (inverted)	ATGCAAATTA	
Synthetic oligonucleotide	GATCCATGCAAAT-AGGGTG GTACGTTTA-TCCCACCATG	

Comparison of part of the U2 gene promoter with sequences found in a human U1 gene¹² and a *Xenopus laevis* U1 gene³⁰. They are compared with sequences found in the 5' flanking region of Immunoglobulin genes of mice and men^{20,21} and of the SV40^{21,22} and Immunoglobulin enhancers. The numbers on the right indicate the distance from the transcription initiation site. The sequence of a synthetic oligonucleotide, designed to test the functional importance of the conserved sequence is also shown. The *Xenopus* U2 sequence is extended to the left to indicate the extent of sequence conservation between *Xenopus*¹⁰, rat²⁷ and human^{9,16} U2 gene promoter sequences. This extended homology is not found in the U1 genes so far sequenced.

immunoglobulin heavy-chain gene enhancer⁸.

To test directly whether this homology is functionally significant, two 19-b complementary oligonucleotides, corresponding to the 14 b between -269 and -256 of the U2 promoter flanked by *Bam*HI linker ends (Table 1), were synthesized. This oligonucleotide duplex was then inserted into two mutants, b and d, both of which lack the distal sequence element. The site of insertion in mutant b, -242 (Fig. 3A), was such that the oligonucleotide was restored close to its original position. The sites of insertion into mutant d were at -82 in the 5' flanking region and +36 in the 3' flanking region (Fig. 3A). The orientation of insertion was determined by DNA sequencing and the clones designated 242+, 242-, 82+, 82-, 3'/36+ and 3'/36-, a plus sign indicating that the insert had the same orientation as in wild-type U2, a minus sign that the insert was inverted (represented by arrows in Fig. 3A). We then coinjected these DNAs with an equal concentration of either maxigene M (Fig. 1A), which has a complete promoter, or a new maxigene construct called dM which was made by joining the promoter of mutant d (Fig. 1A) to the coding region of the maxigene. Mutant dM therefore lacks the distal sequence element. Figure 3B shows the results of competition experiments with this gene. On coinjection, mutants d and dM are equally transcribed (Fig. 3B, lane 2), as are mutants b and dM (not shown). This is expected as these clones all lack the distal sequence element but retain the proximal sequence element. On coinjection of U2 and dM, only U2 transcripts are seen (Fig. 3B, lane 3), because U2 contains the distal sequence element. Mutants 242+ and 242- both show intermediate behaviour, being expressed to a much greater extent than dM regardless of the orientation of insertion of the synthetic oligonucleotide, but a small amount of dM transcription is still detectable (Fig. 3B, lanes 4, 5). These results indicate that insertion of the 14-b synthetic oligonucleotide, corresponding to part of the U2 promoter (Fig. 3A), into a site close to its natural position, results in partial complementation of distal sequence element function. This complementation is independent of the orientation of the insertion of the oligonucleotide despite the fact that it shows no rotational symmetry. To test whether the oligonucleotide would function independently of the position of insertion we next coinjected dM with clones 82+, 82-, 3'/36+ and 3'/36-. The result in Fig. 3B, lane 6 shows that clone 82+ competes efficiently with dM (more efficiently than either 242+ or 242-). The transcript produced

Fig. 3 The *Xenopus* U2 gene. The 188-bp coding region is indicated by the interrupted black rectangle. The positions of conserved sequences representing at least parts of the distal and proximal promoter sequence elements are shown. The TCTCCNNATG sequence was described²⁹ as a sequence element conserved in all *Xenopus* U snRNA genes so far analysed. The arrows above -242, -82 and +36 represent the position and orientation of insertion into the flanking sequences of a synthetic oligonucleotide described in the text and Table 1. **B**, Insertion of a 19-bp synthetic oligonucleotide restores distal sequence element function in an orientation independent manner. Mutant *dM*, constructed by combining the promoter of mutant *d* with the coding sequence of the maxigene through the *Nco*I site at -55, was coinjected with either U2, mutant *d* or a series of clones constructed by inserting a 19 bp synthetic oligonucleotide duplex into mutants *b* and *d* and termed 242+, 242-, 82+, 82-, 3'/36+ and 3'/36- depending on the position and orientation of insertion of the oligonucleotide (Fig. 3A). A plus sign indicates that the oligonucleotide is in the same orientation as in U2-5, a minus sign that it is in the inverse orientation. The two DNAs were coinjected at equal concentration. Lane 1, control oocyte; lane 2, *dM* + *d*; lane 3, *dM* + U2; lane 4, *dM* + 242+; lane 5, *dM* + 242-; lane 6, *dM* + 82+; lane 7, *dM* + 82-; lane 8, *dM* + 3'/36+; lane 9, *dM* + 3'/36-. The U2 and *dM* transcript sizes are indicated. Equal transcription of *dM* and the competitor clone indicates that neither clone can stably bind to a limiting transcription factor. Excess of transcription over *dM* indicates that the competitor clone can bind the factor required for the formation of stable transcription complexes.



from this mutant is slightly longer than U2, which probably results from the close proximity of the distal and proximal sequence elements in this construct affecting the accuracy of transcription initiation. Mutant 82- is transcribed equally to *dM* (Fig. 3B, lane 7) indicating that at this position insertion of the oligonucleotide in inverted orientation has no effect on promoter efficiency. Similarly, insertion of the oligonucleotide in either orientation into the 3' flanking region in clones 3'/36+ and 3'/36- has no effect on promoter efficiency (Fig. 3B, lanes 8, 9). These results were confirmed by testing the oligonucleotide-containing genes against maxigene *M* (Fig. 1A). 242+, 242- and 82+ could all compete to a limited but significant extent with maxigene *M*, whereas 82-, 3'/36+ and 3'/36- could not (data not shown).

Insertion of the synthetic oligonucleotide in either orientation near to its natural position (constructs 242+ and 242-) or 170 base pairs nearer to the cap site in the normal orientation (82+) therefore results in partial restoration of the competitive ability of mutants *b* and *d*. The reason for the restoration being incomplete is probably because the 14 bp of promoter sequence inserted do not represent the entire distal sequence element, but only part of it. In this context the behaviour of mutant *a* in competition with the maxigene is of interest. This mutant, deleted from -358 to -276, to within 7 bp of the homology shown in Fig. 3A, competes less efficiently than U2 with the maxigene (Fig. 1C compare lane *M* + *a* with lane *M* + U2). An explanation for this would be that part of the distal sequence element has been removed in this mutant.

The independence of orientation and, to some extent, of position of insertion of the oligonucleotide suggests that the distal sequence element is enhancer-like. It has been reported previously that viral enhancers are not completely position independent^{2,3}. As the oligonucleotide seems to represent only part of the promoter element this might mean that it would be more subject to effects of the sequences flanking its insertion site. The fact that 82+ competes more efficiently than either 242+ or 242- with both maxigene and mutant *dM* (compare lane 6 with lanes 4, 5 in Fig. 3B) suggests that such position effects can be positive as well as negative.

The *Xenopus* U2 gene promoter contains two elements necessary for maximal transcriptional activity in *Xenopus* oocytes. The distal of the two elements is required for full promoter efficiency, while the proximal sequence element is

essential for transcription. The bipartite nature of the U2 promoter is consistent with results obtained by other laboratories working with U snRNA gene promoters^{9,15,23,24}.

The formation of stable transcription complexes has been shown to be an important step in transcription by RNA polymerases I and III¹⁷⁻¹⁹. In the case of RNA polymerase III transcription the DNA sequences necessary for stable complex formation are internal to the genes¹⁷. The DNA element necessary for stable RNA polymerase I complexes is an enhancer, that is, is orientation and position independent²⁵. The results presented here show that the formation of stable transcription complexes is also a step in RNA polymerase II transcription *in vivo*. In addition the DNA sequence element required for their formation acts independently of orientation, that is, it has some of the properties of an enhancer. This suggests that the function of enhancers in general is to promote the formation of stable transcription complexes, a hypothesis that could be tested experimentally with better characterized enhancers in other gene systems, such as SV40 or polyoma. The U2 distal sequence element function is partly restored by insertion of a synthetic oligonucleotide containing only 14 base pairs of U2 promoter sequence. This sequence does not display rotational symmetry, and does not appear to be repeated within the U2 tandem repeat unit. Surprisingly, the first 8 b of these 14 are identical to a sequence element found roughly 60 base pairs 5' of the cap site of mammalian immunoglobulin heavy-chain genes^{20,21} (Table 1). This element is found in the same position, but in the opposite orientation, in the promoter of immunoglobulin light-chain genes^{20,21}. This sequence also has a counterpart in the SV40 enhancer^{21,22} (Fig. 3A), and is also present, in the opposite orientation, in the immunoglobulin heavy-chain enhancer⁸ (Fig. 3A). In fact the G residue located in this sequence is protected against dimethyl sulphide modification in cells of the B lineage, but not in other cell lines⁸. This suggests that this sequence, within the immunoglobulin heavy-chain enhancer, interacts with a factor in B cells, and this is likely to be important in bringing about the cell-type specific expression of the immunoglobulin genes. These data support the notion that this element is a general, orientation independent promoter element. The eight absolutely conserved bases may represent the core of the element, whose activity could be modified by non-conserved sequences in the immediate vicinity. It may be that the distinction between enhancers and other upstream promoter elements will

become more tenuous as we understand more about how they function. Note that the first orientation-independent promoter element was found when a restriction fragment upstream of the sea-urchin H2A histone gene was inverted²⁶.

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Note added in proof: Recent experiments carried out in collaboration with Angelo Tognoni and Walter Schaffner have shown that in CV1 (Monkey) cells the *Xenopus* U2 distal sequence element can functionally replace the SV40 enhancer to allow replication and T-antigen expression in SV40 mutants lacking the SV40 enhancer.

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Amplification of *inter-Alu* extrachromosomal DNA during cellular ageing: retraction and explanation

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We reported previously^{1,2} the age-dependent appearance of extrachromosomal circular DNA bands hybridizing to a human DNA fragment ('*inter-Alu*'), isolated from a genomic cluster of *AluI* repeats³. Such bands appeared or increased at late passage in four out of six human fibroblast strains (six out of nine cell expansions); moreover, all DNAs (9/9) obtained from peripheral lymphocytes of aged donors, but none (0/8) from young donors, revealed a non-genomic *inter-Alu* band at ≈ 4.8 kilobases (kb)^{1,2}. Subsequent data extended these numbers to 16/24 aged donors compared with 0/18 young donors⁴. These results were interpreted as evidence of age-dependent DNA rearrangement in normal human cells^{1,2}. We now report that the 'extra' bands were of microbial origin, although clearly occurring in an age-dependent manner.

The hybridizations reported^{1,2,4} primarily used the entire *inter-Alu*-containing plasmid, p λ H15A, as ³²P-DNA probe. Gel-purified insert DNA also hybridized to the extrachromosomal species, which was then believed to be an adequate control for plasmid contamination. Such hybridization, however, could still be due to residual traces of vector DNA, "invariably present in [insert] probes . . . even after two cycles of electrophoretic purification" (ref. 5). Control hybridizations against many of the same

DNA preparations, with a pCR1 fragment or pBR322 plasmid containing other inserts, revealed no extra bands. However, when we obtained the parental vector for *inter-Alu*, pACYC184, we observed hybridization of this probe to the age-dependent bands in both fibroblast and lymphocyte DNA samples, indicating that such bands must be due to low levels of microbial plasmid DNA ($\approx 10^{-6}$) in our human DNA samples. We discussed the dangers of microbial contamination¹, but were misled by unwarranted confidence in the controls, the pervasive extent and polymorphic nature of the contamination, and its remarkable age dependence.

In attempting to account for our data, we have considered the possibility of deliberate contamination and regard it as extremely unlikely. Inadvertent operator contamination, however, is difficult to reconcile with the marked age bias observed, in particular for the multiple fibroblast DNA bands seen in many independent samples, prepared by six persons over a 2-yr period. Biological contamination is more plausible in the light of recent reports of microbial plasmid DNA in biopsy and necropsy tissue samples and in body fluids⁵⁻⁷. Our preliminary evidence indicates that plasmid-homologous DNA can be detected in filter-sterilized serum and trypsin, two common reagents for cell culture. Thus, the correlation between culture age and plasmid bands may reflect cumulative exposure of fibroblasts to plasmid DNA.

We apologize for any inconvenience to other investigators resulting from this error. Our experience, along with other reports^{5,6,8}, underscores the danger of systematic plasmid contamination of DNA samples, and the necessity for control hybridizations using the appropriate vector alone as probe.

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***In vitro* reactivation of anaphase spindle elongation using isolated diatom spindles**

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A key step for analysing the mechanochemistry of mitosis would be the isolation of a functional spindle capable of anaphase chromosome movement *in vitro*. Although Mazia and Dan first isolated spindles in 1952¹, with one or two possible exceptions²⁻⁴, isolated spindles are non-functional (reviewed in ref. 5). An alternative approach has used permeabilized cells to study anaphase chromosome movement⁶⁻¹⁰, but these preparations are biochemically and morphologically complex, and hence difficult to analyse. We describe here a simple procedure for isolating diatom spindles which are capable of anaphase spindle elongation *in vitro*. With addition of ATP, the two half-spindles slide completely apart, with concomitant decrease in the zone of overlap. Electron microscopy reveals decreased numbers of microtubules throughout the spindle after ATP addition and confirms the complete absence of structures beyond the spindle poles. These results are inconsistent with theoretical models of mitosis which suggest that spindle poles are pushed apart by microtubule growth¹¹, are pulled apart by external forces applied to the poles¹²⁻¹⁵, or are

released from tension generated during spindle formation¹⁶. The results are consistent with models that postulate mechanical interactions in the zone of microtubule overlap as a factor in spindle elongation^{17,18}.

Diatom spindles are important model systems for studying the morphological changes associated with anaphase chromosome movement because the central spindle microtubules are arranged in paracrystalline arrays and the microtubules associated with chromosome-to-pole movement (anaphase A) are spatially separated from the microtubules associated with spindle elongation (anaphase B)^{19,20}. Morphometric analysis has shown that the diatom central spindle is constructed of two half-spindles which exhibit specific near-neighbor interactions in the zone of overlap²¹. This microtubule overlap zone, which is visible even by light microscopy, decreases as the central spindle elongates during anaphase B²².

The centric diatom *Stephanopyxis turris* was selected as starting material because it can be grown in large quantities in a simple medium and its cell cycle is easily synchronized (L. Wordeman, K.L.Mc.D and W.Z.C., in preparation). Following homogenization, spindles are isolated by a two-step procedure consisting of filtration to remove cell walls and low-speed centrifugation of spindles onto coverslips (Fig. 1). The central spindle is readily detected as a phase-dense bundle of fibres embedded in a tangled mass of chromatin (Fig. 1a). In polarization optics, the central spindle is a highly birefringent structure with a prominent zone of microtubule overlap, showing increased birefringence, which is 15–25% of the total spindle length (Fig. 1b, Table 1).

Addition of 1 mM ATP by perfusion leads to an increase in

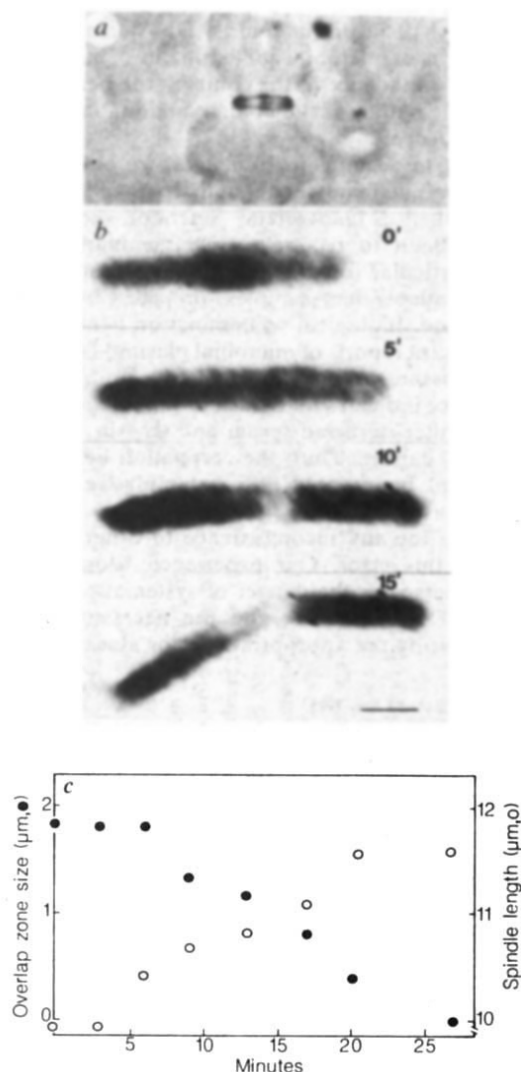


Fig. 1 Changes in spindle structure after reactivation as monitored by light microscopy. *a*, An isolated spindle viewed with phase optics. Note the prominent phase-dense overlap zone and the tangled mass of chromatin. *b*, An isolated spindle viewed with polarization optics with negative compensation, just before ATP addition (0 min) and after addition of 1 mM ATP (at 5 min, 10 min, 15 min). Note the decrease in the birefringence in the zone of microtubule overlap during spindle elongation. There is further loss of birefringence from the midzone even after elongation is complete. The spindle elongated at a rate of $0.3 \mu\text{m min}^{-1}$. Scale bar, $2 \mu\text{m}$. *c*, Change in extent of birefringence of the zone of microtubule overlap (●) during spindle elongation (spindle length; ○) on addition of 1 mM ATP at time 0. Midzone birefringence continues to decrease after elongation is complete. The rate of elongation in this example, which differs from *b*, is $\sim 0.1 \mu\text{m min}^{-1}$. Normal anaphase spindle elongation occurs at a rate of $1\text{--}2 \mu\text{m min}^{-1}$ and the increase in spindle length is much greater than the size of the overlap zone. Some of this length increase must be due to microtubule growth (data not shown).

Methods. Cells were grown in f/2 medium³⁰ in a suspension culture on 6-h-day, 18-h-night schedule at 19°C . Three hours before collection $2 \times 10^{-8} \text{ M}$ nocodazole was added and 20 min before collection, cells were placed on $60\text{-}\mu\text{m}$ mesh Nitex filters and washed extensively to remove the drug. Cells were collected by filtration at the height of the division peak which occurred $1\frac{1}{2}\text{--}2 \text{ h}$ after night began. Cells were resuspended in homogenization medium at room temperature (50 mM PIPES, pH 7.0, 40 mM sodium glycerophosphate, 10 mM MgSO_4 , 10 mM EGTA, 0.2% Brij 58, 30% glycerol, 1 mM dithiothreitol (DTT) and inhibitors of proteolysis³¹, then homogenized on ice with two strokes of a Kontes Dounce homogenizer, B pestle. The homogenate was filtered once through $60\text{-}\mu\text{m}$ mesh and once through $25\text{-}\mu\text{m}$ mesh Nitex filters, then centrifuged for 20 min, 0°C at $2,000 \text{ g}$ onto coverslips. Coverslips were mounted on slides, using strips of Parafilm as spacers, waxed down with Valap⁷, and then perfusion was performed as described previously for lysed cells⁸. Reactivation medium consisted of 50 mM PIPES pH 7.0, 5 mM MgSO_4 , 10 mM EGTA, 1 mM ATP, 1 mM DTT, with or without 7.5% glycerol. The progress of reactivation was monitored with Zeiss polarization optics, $40\times$ Nikon rectified lens, Brace-Kohler $\lambda/30$ compensator and a DAGE-MTI low light level television camera connected to a Panasonic time-lapse video recorder. Two KG1 heat filters, a 1.5-inch column of saturated copper sulphate solution, and a narrow-pass green interference filter were placed between the 100-W mercury light source and the condenser to minimize damage to the spindle. The isolation medium was designed to stabilize the microtubule components of the spindles, but it was necessary to include protease and phosphatase inhibitors to obtain reliable reactivation of spindle function. Although yield is low, the coverslips are covered with hundreds of spindles at a similar stage of mitosis (metaphase to early anaphase); the only contaminants present are interphase nuclei, chloroplasts and glass cell wall fragments.

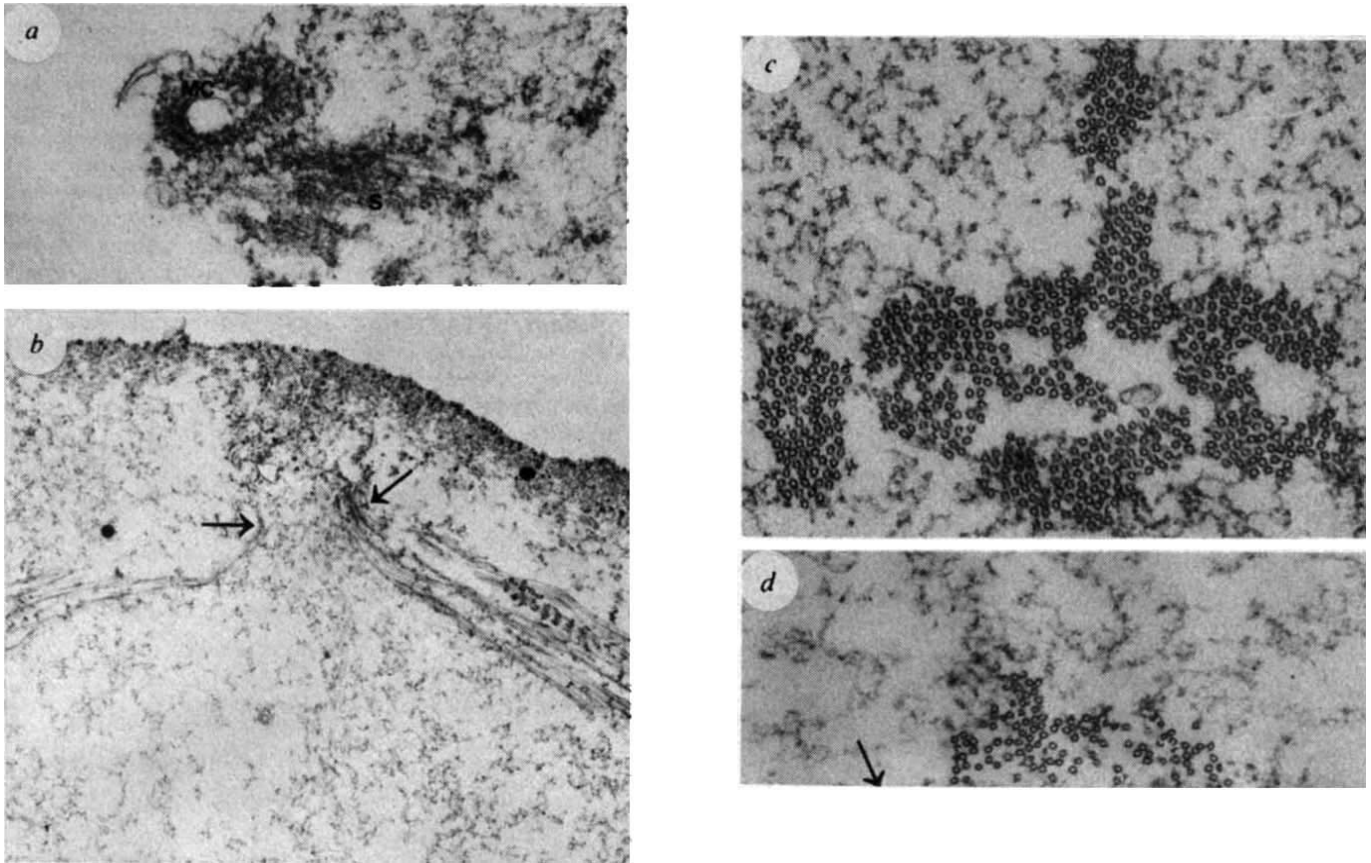


Fig. 2 Electron microscopy of isolated spindles with (*b, d*) and without (*a, c*) added ATP. *a*, Spindle pole structure showing the mitotic centre (MC) or centrosome at the end of the obliquely sectioned spindle (S). No cytoplasmic structures extend beyond the pole. $\times 28,500$. *b*, Longitudinal section through the middle of an isolated spindle after 10 min in 1 mM ATP. The few remaining MTs in this region are abnormally curved (arrows). $\times 11,000$. *c*, Cross-section through the overlap region in an isolated anaphase spindle (minus ATP). There are 623 hexagonally packed microtubule profiles in this section. $\times 41,000$. *d*, Cross-section through the overlap region in an ATP-treated spindle of comparable mitotic stage with the one in Fig. 2*c*. There are 108 microtubule profiles in this section. This spindle is very close to where the coverslip was (arrow) before it was dissolved with HF before sectioning. $\times 41,000$.

Methods. Isolated spindles attached to coverslips were fixed with 0.5% glutaraldehyde in f/2 medium³⁰ for 30 min at 23 °C (unless otherwise stated, all remaining steps were at 23 °C). Spindles were rinsed for 5 min in f/2, then 5 min in PBS (physiologically buffered saline: 137 mM NaCl, 2.68 mM KCl, 8 mM $\text{Na}_2\text{HPO}_4/\text{H}_2\text{O}$, 1.47 mM KH_2PO_4) at pH 7.3, fixed in 0.2% OsO_4 + 0.3% $\text{K}_3\text{Fe}(\text{CN})_6$ in PBS for 15 min at 4 °C, rinsed twice in PBS for 5 min each, rinsed in distilled H_2O for 5 min, incubated in 2% aqueous uranyl acetate for 90 min, dehydrated in acetone by 10% steps for 10 min each, and embedded in Epon-Araldite.

spindle length and a concurrent decrease in the magnitude and extent of birefringence in the zone of microtubule overlap (Fig. 1*b, c*). Within 5 min after ATP addition, spindles begin to elongate at a rate of 0.1–0.4 μm per min for several minutes. After spindle elongation is complete, the birefringence in the spindle midzone continues to decrease. In the example shown in Fig. 1*b*, the two half-spindles have become disengaged and the extent of birefringence of each half-spindle has shortened by $\sim 1 \mu\text{m}$.

For all of the spindles studied on-line by video microscopy (Table 1), we find that the increase in spindle length is about the same as the length of the microtubule overlap zone and that spindle midzone birefringence always continues to decrease after elongation is complete. The sample size in Table 1 is necessarily small because the isolated spindles are easily damaged by the manipulations required for on-line observations. It is easier to study the physiology of spindle elongation using populations of spindles (see Table 2). After incubation for 10 min in 1 mM ATP, >85% of the spindles on a coverslip show distinct midzone birefringence changes. Those spindles which display reduced birefringence are significantly longer than control (minus ATP) spindles or spindles which do not have reduced midzone birefringence.

Longitudinal sections and serial cross-sections of *in vitro* spindles show that the mitotic centre is retained during isolation (Fig. 2*a*) and that no other structures extend beyond the end of the isolated central spindle. The overlap zone is the region most drastically altered by ATP. Normally the most well-ordered part

of the spindle^{19–21}, the overlap is in complete disarray after 10 min in 1 mM ATP (Fig. 2*b*). Isolated spindles (minus ATP) were similar to *in vivo* spindles in the number of microtubules per half-spindle (~ 300) and extent of microtubule overlap at early anaphase ($\sim 20\%$). After 10 min in 1 mM ATP, the number of microtubules present drops to about 110–120 per half-spindle, and relatively few microtubules are found in the overlap region (Fig. 2*d*) compared with control spindles (Fig. 2*c*).

The newly formed gap between the half-spindles is not due just to microtubule depolymerization. The microtubules in the isolated central spindles are very stable and the birefringence pattern shown in Fig. 1*b*, 0 min, will persist for several hours in the absence of ATP. Addition of nonspecific agents which promote microtubule depolymerization, such as high salt, do not lead to gap formation, but rather to a decrease in overall birefringence and some spindle shrinkage. This shows that ATP addition leads not only to spindle elongation but also to a defined pattern of microtubule disassembly, starting from the midzone and working poleward. We suggest that the mechanochemical events leading to spindle elongation and the ATP-induced lability of microtubules *in vitro* are closely related. One possibility is that there is a discrete cap on the end of every microtubule in the overlap zone (the plus end^{23,24}) that regulates microtubule assembly–disassembly in an ATP-dependent manner. Alternatively, or in addition, lateral interactions between overlapping microtubules may stabilize them²⁰, and as the two half-spindles separate, giving less overlap, the microtubules begin to depolymerize. Either suggestion would be consistent

Table 1 *In vitro* spindle elongation monitored by video microscopy

Original spindle length (μm)	Final spindle length (μm)	Length change (μm)	Length of initial zone of microtubule overlap (μm)
9.3	11.0	1.7	1.8*
10.1	12.0	1.9	2.2*
6.3	7.5	1.2	1.1*
8.8	10.5	1.7	2.0†
8.3	10.0	1.7	2.0†
9.6	11.7	2.1	2.1†

* There was a distinct gap between half-spindles at the end of experiment.

† Spindle midzone was reduced in birefringence to same level as the rest of the half-spindle and the midzone was thinner or collapsed.

Table 2 Average spindle lengths of experimentally treated populations of spindles

Treatment	n	Average spindle length (μm)	Size of zone of microtubule overlap (μm)	Change in average spindle length (μm)
No ATP control	25	8.4 ± 0.9	1.9 ± 0.2	
Plus 1 mM ATP:				
Reduced overlap zone birefringence	26	$10.7 \pm 0.8^*$	†	2.3‡
No change in overlap zone birefringence	5	8.2 ± 0.6	1.7 ± 0.2	-0.2

Coverslips prepared as described in Fig. 1 legend were incubated for 10 min at room temperature in reactivation medium with or without ATP, then 0.5% glutaraldehyde was added. The lengths of individual spindles were measured off the TV monitor. All spindles on a transect of the preparation were used for these measurements, except it was necessary to disregard five or six spindles whose poles were not in good focus or were obscured by debris.

* The difference between the means (compared with no ATP) was statistically significantly different at $P < 0.001$ using Student's *t*-test.

† The average size of the gap between the two half-spindles was $0.7 \pm 0.3 \mu\text{m}$.

‡ As compared with average spindle length of control (minus ATP) spindles.

with the directed pattern of microtubule disassembly observed during telophase in the diatom *Pinnularia*²⁵ or after ultraviolet microbeaming of diatom spindles²⁶.

Anaphase chromosome movement is a complex process involving several distinct mechanochemical events (reviewed in refs 27, 28). Our observation of a loss of birefringence in the zone of microtubule overlap during reactivation of spindle elongation suggests that microtubule sliding is the key mechanochemical event that occurs during anaphase B. Ultraviolet microbeaming experiments on living dividing diatoms demonstrate that anaphase B *in vivo* also depends on the generation of sliding forces in the zone of microtubule overlap²⁹. Several investigators have suggested that dynein-like interactions between interdigitating sets of microtubules in the overlap zone may be responsible for spindle elongation^{17,18,20}. Although we cannot identify the motor, the requirement for ATP and inhibition of elongation by $10 \mu\text{M}$ vanadate (data not shown) suggests that a dynein-like 'ATPase' may be involved. The observation that similar conditions affect spindle elongation in lysed mammalian cells¹⁰ suggests that an analogous mechanism may be important for anaphase B in a wide variety of cells.

The forces responsible for spindle elongation *in vitro* could not have been generated by microtubule polymerization¹¹, as no tubulin is present during reactivation. However, *in vivo* in this diatom and many other cells, microtubule growth is an integral part of anaphase B and leads to an increase in spindle length much greater than can be generated by microtubule rearrangements alone. The autonomy of the central spindle and

the lack of attachment of the spindle poles to any other cytoplasmic structures beyond the poles eliminates the possibility that the half-spindles are pulled apart by astral microtubule interactions with cytoplasmic components¹²⁻¹⁵. Although it has been suggested that spindle poles are self-propelled¹³, these phenomena may have more to do with nuclear migration and repositioning at the end of cell division than with anaphase B. Finally, it has been suggested that tension generated during spindle formation and released during chromosome-to-pole movement may be responsible for spindle elongation¹⁶. As little or no chromosome-to-pole movement occurs during spindle reactivation, this mechanism is also inconsistent with our observations.

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β -Hairpin families in globular proteins

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β -Hairpins, one of the simplest supersecondary structures, are widespread in globular proteins, and have often been suggested as possible sites for nucleation¹. Here we consider the conformation and sequences of the loop regions of β -hairpins by analysing proteins of known structure. We find that the 'tight' β -hairpins, classified by the length and conformations of their loop regions, form distinct families and that the loop regions of the family members have sequences which are characteristic of that family. The two-residue hairpin loops include almost entirely I' or II' β -turns, in contrast to the general preference for type I and type II turns^{2,3}. These findings are being used to help define templates or consensus sequences to be incorporated into our existing supersecondary structure prediction algorithm^{4,5}. This information can also be used in model-building homologous proteins.

Fig. 1 *a*, Distribution of the number of residues in the loop region for 107 β -hairpins. *b*, Schematic diagram of a β -hairpin. Data derived from the 62 proteins included in the Kabsch and Sander dictionary of secondary structure⁶. Loop residues are defined as those not participating in the antiparallel β -ladder⁶, which forms the hairpin. Residue i participates in the ladder if both its NH and CO groups form the standard antiparallel H-bonds, or if adjacent residues CO_{i-1} and NH_{i+1} form these bonds. If the first and last residue of the loop are linked by one hydrogen bond (NH_{i+1} to CO_{i-1}), this is denoted by an asterisk in Table 1. This definition is unequivocal and in practice is simple to apply to the Kabsch and Sander output. The ladder must include at least two residues in each strand, and only loops which do not include a helix or another strand are counted. For homologous loops (that is, >50% amino-acid identity), only one family member was included.

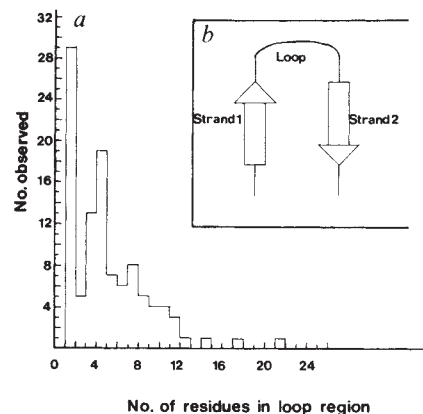
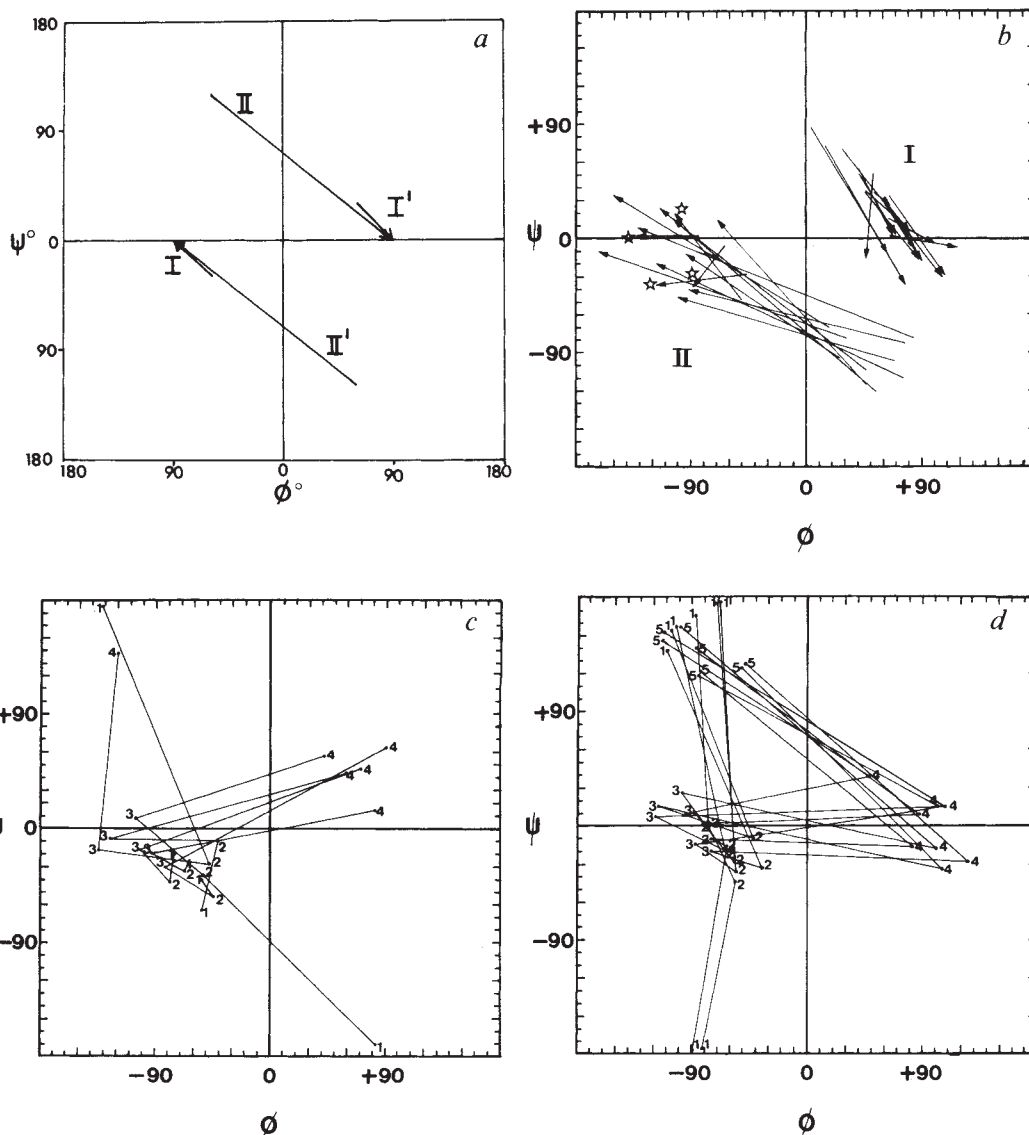


Fig. 2 Conformation of loop regions shown by plotting ϕ , ψ values for the loop residues. *a*, Classical β -turn conformation types I and II and their mirror images I' and II' taken from Chou and Fasman². These are defined by the ϕ , ψ values of the two central residues of the turn (usually referred to as residues 2 and 3) which in the context of, say, a two-residue hairpin, will be described as residues L1 and L2. The ϕ_{L1} , ψ_{L1} , ϕ_{L2} , ψ_{L2} values plotted are type I = -60° , -30° , -90° , 0° ; type I' = $+60^\circ$, $+30^\circ$, $+90^\circ$, 0° ; type II = -60° , 120° , 90° , 0° ; type II' = 60° , -120° , -90° , 0° . Since type III turns do not form a group distinct from the type I turns¹⁶, they have been omitted. Arrowheads denote the L2 ϕ , ψ value. *b*, ϕ , ψ Plot for the 29 two-residue hairpin loops given in Table 1. For each loop the ϕ , ψ values of residue L1 are connected by a line to the ϕ , ψ values of residue L2, with the arrowhead denoting the L2 position. Types I' and II' turns predominate, with only four type I turns, denoted by stars, and no type II turns, observed. *c*, ϕ , ψ Plot for the six four-residue hairpin loops with type I turns given in Table 1a. For each loop the ϕ , ψ values of residues L1, L2, L3 and L4 are plotted and joined together sequentially. Five of these hairpins also have residue L4 in the ++ quadrant of the ϕ , ψ plot. The ϕ , ψ angles calculated by averaging over the four similar loops for residues L1 to L4 are -62° , -38° ; -58° , -35° ; -101° , -19° ; 75° , 42° . *d*, ϕ , ψ Plot for the eight five-residue hairpin loops which form a family (see Table 1a). For each loop the ϕ , ψ values of residues L1, L2, L3, L4 and L5 are plotted and joined together sequentially. Note the type I turn for residues L2 and L3 and the positive ϕ values for residue L4. The standard ϕ , ψ angles for residues L1 to L5, calculated by averaging over the eight hairpins in the family, are -90° , 161° ; -56° , -24° ; -92° , 2° ; 95° , 2° ; -85° , 136° .



A β -hairpin involves two consecutive β -strands that lie antiparallel and are linked by hydrogen bonds (Fig. 1). The connecting loop region may or may not include a classical β -bend^{2,3}. To analyse the number of residues in the loop region, we used the non-subjective assignments of β -strands and coil regions given for the 62 proteins included in Kabsch and Sander's

dictionary of secondary structures⁶. Thirty-nine proteins had β -hairpins, giving a total of 107 hairpins. Loop residues (referred to as L1, L2, ...) are defined as those not participating in the antiparallel β -ladder which forms the hairpin (see Fig. 1 legend). The residues of the hairpin strands are denoted by ... -B3, -B2, -B1 for the N-terminal strand and ... +B1, +B2,

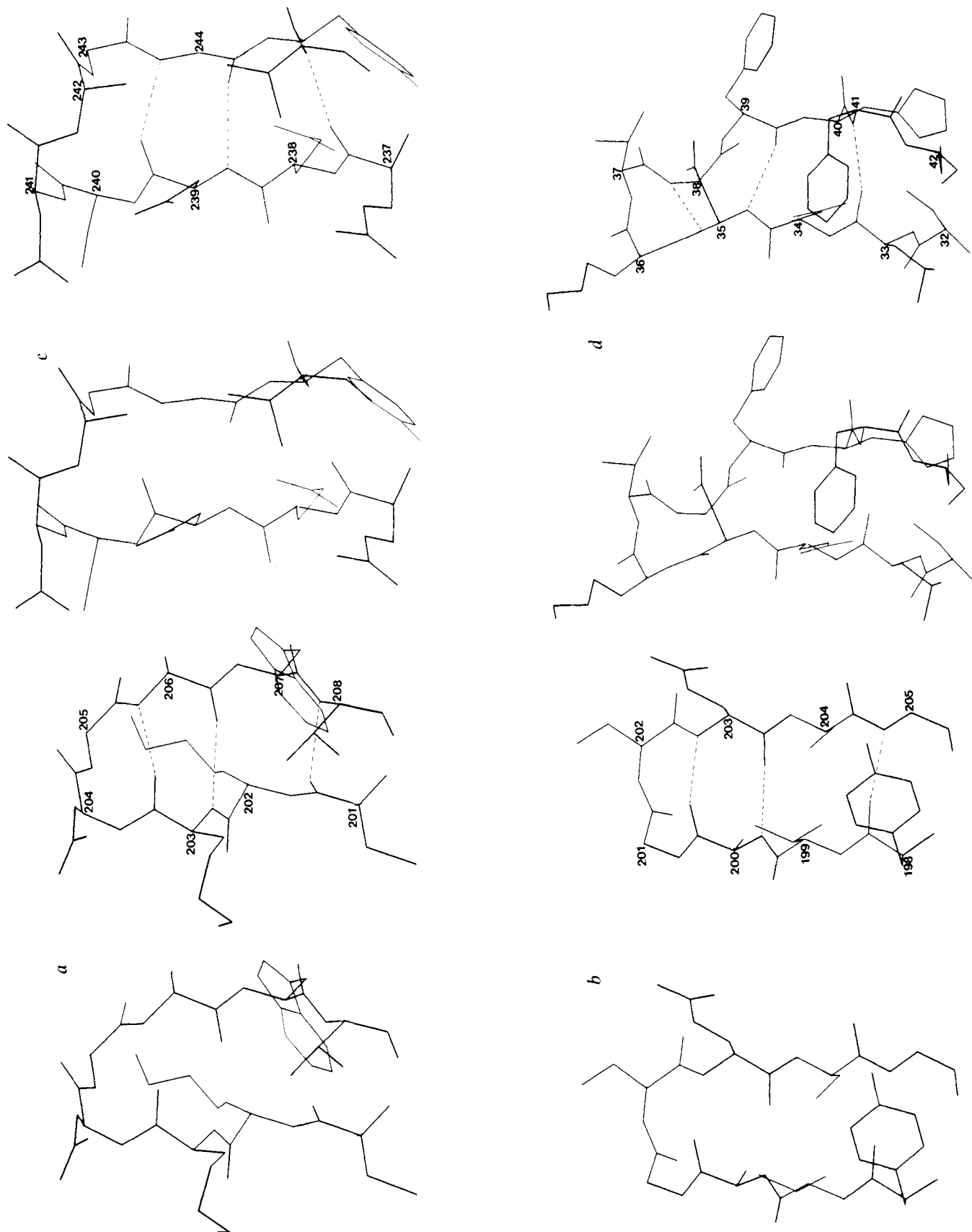


Fig. 3 Stereo diagrams of representative structures for the two-, four- and five-residue hairpin-loop families. *a*, Two-residue hairpin loop with a type I' β -turn: γ -chymotrypsin A, residues 201–208. *b*, Two-residue hairpin loop with a type II' β -turn: penicillopepsin, residues 198–205. *c*, Four-residue hairpin loop with a type I β -turn: penicillopepsin, residues 237–246. Note how the amide NH groups of residues L2, L3 and L4 point inwards towards the centre of the turn and to the carbonyl oxygen of the residue immediately before the loop (–B1). The side chain of this residue may help to neutralize the cluster of NH groups. Some of these bends have been described previously by Isogai *et al.*¹⁷ and Richardson¹⁶. *d*, Five-residue hairpin loop with a type I β -turn and a G1 β -bulge: γ -chymotrypsin A, residues 32–42. This structure is reminiscent of the four-residue family in that the nitrogens of residues L3, L4 and L5 all point inwards towards the carbonyl of residue L1, and an aspartyl oxygen is often observed nearby.

Table 1 β -Hairpin loops in distinct structural families (A) and miscellaneous structures (B)

A								
β -Hairpins with two-residue loops								
Protein	Type I'		Type II'		β -Hairpins with four-residue loops		β -Hairpins with five-residue loops	
	Residue nos	Sequence	Residue nos	Sequence	Residue nos	Sequence	Residue nos	Sequence
Actinidin	170-177	GTEGGVDY						
Superoxide dismutase	21-28	EAKGDTV						
Tosyl elastase	201-208	CLVNGQYA	35-39	YRSGSSWA				
Cytochrome b_5	23-30	LILHYKVY						
Staphylococcal nuclease	92-99	IYADGKMV						
D-Glyceraldehyde 3'-phosphate dehydrogenase	64-71	LVVDGKKI	299-306	QLSKTFVK				
Immunoglobulin Fab NEW (light and heavy chain)	58-65	KMEDGALV						
Immunoglobulin REI			L64-L71	SKSGSSAT	H202-H211	VNHKPSNTKV	H49-H59*	GYVFYHGTS
Liver alcohol dehydrogenase			L89-L98	SYDRSLRV				
Penicillopepsin			65-72	SGSGTDYT				
	21-28	VTIGGTTL	130-137	FTCRGKPI				
	90-97	VTVGGVTA	198-205	YTAGSQSG	237-246	QQDSNAGGYV	72-82*	SISYGDGSSAS
	258-265	VSISGYTA						
γ -Chymotrypsin A	201-208	CKKNGAWT					32-42*	SLQDKTGPHFC
S-subtilisin inhibitor	84-99	GVWQGRV						
Phospholipase A ₂	76-83	SCSNNEIT						
α -Lytic protease	66-85	ARIGGAVV	48-51	VTRGATKG				
			118-122	VANGSSFFV				
Prealbumin					16-25	VLDVARGSPA	33-43*	FRKAADDTWEP
Strep. Griseus Protease A	48-51	VSVNGVAH	199-210	LFAGSTAL				
Lysozyme HEW					51-60	TDYGILQINS		
Lysozyme T ₄							17-27*	IYKDTGEYITI
Papain					164-173*	VGYPNYILI		
Trypsin inhibitor					22-31	FYNKAGLCQ		
Carboxypeptidase A							38-48*	IGRSYEGRIPIY
Thermolysin							9-19*	VGRGVLGDQKN
Ovomucoid (3rd domain)							22-32*	PVCGSDNKTYS
B								
β -Hairpins with two-residue loops								
Protein	Type I		β -Hairpins with four-residue loops		β -Hairpins with five-residue loops		β -Hairpins with five-residue loops	
	Residue nos	Sequence	Residue nos	Sequence	Residue nos	Sequence	Residue nos	Sequence
Cytochrome b_5	15-22	HNNKSTW						
γ -Chymotrypsin A	46-53	LINENWVV						
α -Lytic protease			120-129	TANYAEGAVR	30-43*	EYSINNASLCS		
					90-105*	ARVFPNDRAW		
Strep. Griseus Protease A					117-123*	RVLYNGSYQD		
					168-178*	TVNYGSSGIVY		
Prealbumin	110-117	LLSPYSYS						
Neurotoxin B	29-36	WSDFRGTI						
Carbonic anhydrase C			62-71*	ILNDGHAFNV	L166-L176*	PSKQSNKYYAA		
FAB			L196-L205*	QVTHEGSTVE	H172-H182*	AVLQSSGLYSL		
					15-25*	IKAIDGDTVKL		
Staphylococcal nuclease			24-33*	KLMYKGPMT				
Penicillopepsin			275-284	GPSGNGSTCL				
			309-318	VFDSDGPQLG				
Glutathione reductase			422-431	VCANKEEKVV	369-379	TVVFSHPPIGT		
Concanavalin A					146-156*	ATTGTDGNLEL		
Thermolysin					21-31	NTTYSTYYLQ		
S-subtilisin inhibitor					32-34	TLTCAPGPSGT		

Sequences for two-, four- and five-residue loops. A, Sequences for those hairpins which form distinct structural families, whose ϕ , ψ values are plotted in Fig. 2. B, Sequences for the loops with miscellaneous structures. The standard one-letter amino-acid code is used. Residue numbers are taken from Cambridge Protein Data Bank Files¹⁵ given in ref. 6, except for penicillopepsin for which the more recent 2APP file was used.

* Loops with a single hydrogen bonds between the first (NH) and last (CO) residues in the loop.

+B3... for the C-terminal strand. Thus, a two-residue hairpin is described as (...-B3, -B2, -B1, L1, L2, +B1, +B2, +B3...).

The distribution of loop length in these 107 hairpins (Fig. 1) clearly shows the predominance of short loop connections, with 70% of all the observed hairpins having <7 residues in their loop regions. Even more striking is the high number of two-residue loops (29 out of 107) and to a lesser extent the four- and five-residue loops (13 and 19 observed, respectively).

The short loop β -hairpins (two-, four- and five-residue) were analysed further by plotting the main-chain dihedral angles ϕ and ψ for these loops (Fig. 2). The plots show that many of the β -hairpins fall into structural families (Table 1a), with the loop regions folded in specific ways. The two-residue β -hairpins all include one of the classic β -turn conformations categorized as types I-VIII³ (see Figs 2b, 3a, b). In a study on all the turns

found in proteins of known structure, Chou and Fasman² found that 60% were type I + III and 15% were type II (Fig. 2a). The mirror images of these two types were rarely observed, type I' constituting only 3% and type II' 5%. For hairpins, however, the types I' and II' turns predominate. Out of the 29 hairpins, 15 were type I', 10 type II' and only 4 type I. The total absence of type II turns in the hairpins and relative lack of type I turns strongly implies that these turns, which are by far the most commonly observed throughout proteins, are not compatible with an antiparallel β -hairpin. In contrast, their mirror images, which are thought to be energetically less favourable because of steric hindrance³, are clearly preferred in the context of a β -hairpin. Preliminary studies suggest that the type I' turn has the correct twist to match the relative twist which is always observed between adjacent strands⁷ (Fig. 3a). The mirror-image

type I has the incorrect twist and may therefore be unfavourable. In contrast, the types II and II' turns are relatively planar (see Fig. 3b). However, model-building two β -strands with an average twist and standard β -turns on a computer graphics system shows clearly that the types I' and II' turns give acceptable hairpins, whilst the strands rapidly diverge when a type I or II turn is included.

The sequences of the two-residue hairpins are shown in Table 1. As might have been expected from the observed ϕ, ψ plot, 12 of the 15 type I' hairpins have glycine at position L2, while position L1 is dominated by glycine, asparagine and aspartic acid (left-handed helical residues⁸). Similarly, 7 of the 10 type II' hairpins have glycine at position L1, whilst position L2 is usually the polar serine or threonine. The absence of proline is striking.

The 13 four-residue loop hairpins include various turn conformations, with the dominant grouping a family of six structures with a type I turn (Figs 2c, 3c). Four of these hairpins have a particularly similar conformation, with residues L1, L2 and L3 in the α -helical portion of the ϕ, ψ plot and residue L4 (glycine or asparagine) in the $+/+$ quadrant. The tip of these four-residue hairpins is bent over, rather like an erect hooded cobra.

The ϕ, ψ plots for the 19 five-residue loop hairpins showed clearly that one group including eight examples all adopted the same structure (Figs 2d, 3d). This may be described as a type I turn, with a hydrogen bond between the carbonyl residue L1 and the amide of residue L4, and a G1 type β -bulge⁹. This structure demands a glycine at position L4, which is observed in six of the eight sequences, with asparagine and aspartic acid in the other two (Table 1). Of the remaining five-residue loops, three include an L2(CO)-L5(NH) hydrogen bond (although the ϕ, ψ values are different) and four others have glycine at position L4, although they show no structural similarity.

One of the major aims of our study was to search for patterns to aid the prediction of hairpins from sequence. The two-residue loops are strongly selective for amino-acid type. For a type I' turn, L1 = Asn, Asp, Gly; L2 = Gly; for a type II' turn, L1 = Gly and L2 = Ser, Thr or polar. The four- and five-residue loop families have a weaker preference for glycine at position L4. The other residues are usually hydrophilic, as expected for an exposed loop, and often include the classical turn-forming residues.

There is also evidence for distinctive patterns in the residues on either side of the hairpin loop. In addition to the usual alternation of hydrophobic and hydrophilic residues, a matching of residue types across the hairpin is observed. Thus, residues +B3 and -B3 are usually both hydrophobic or both hydrophilic (80% of sequences in Table 1), similarly residues +B2 and -B2 (67%). Such paired residues point in the same direction and are usually therefore in a very similar environment.

These patterns are being incorporated into our template prediction algorithm, which aims to recognize β -hairpins rather than just β -strands⁴. Currently, for the β family of proteins, we

obtain an ~8% improvement in prediction accuracy over the conventional secondary structure prediction algorithms. This is similar to the improvement obtained for the $\beta\alpha\beta$ proteins⁵.

Our results show that the irregular coil regions connecting secondary structures in proteins may include structural and sequence patterns. This limited survey of β -hairpins indicates that the short loop hairpins are highly selective in sequence and can provide a valuable aid to *ab initio* prediction and model-building studies. Insertions and deletions most commonly occur in the loop regions of proteins¹⁰, so that prediction of an unknown structure by homology to a known structure becomes a problem of building the loop regions (for example, renin¹¹). We are currently studying the longer loops and homologous β -hairpins to determine the effects on loop conformation.

In the β -sandwich structures there are essentially only two types of connections between β -strands—a β -hairpin between adjacent H-bonded strands and β -arch between strands on opposite sheets crossing the top of the sandwich. In terms of chain topology, it is critical to differentiate a β -arch from a β -hairpin. Although this will clearly depend largely on the amino-acid composition of the strands (for example, very hydrophobic strands will occupy central positions in the sheet¹²), the sequence of the connecting loop may also be influential. Preliminary studies of β -arches show that their length distribution is grossly different from that of β -hairpins, with a peak for the eight-residue loop. Sequence patterns may also be found for connections between helices^{13,14} and between strands and helices in the $\beta\alpha\beta$ unit. With such information on the loop regions of proteins, the prediction of structure from sequence by template and pattern recognition methods becomes very attractive.

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Molecular Basis of Aging. A.K. ROY and B. CHATTERJEE (eds). Academic: 1984. Pp.400. ISBN 0-12-601060-9. \$29.50, £23.50.

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Neonate Cognition: Beyond the Blooming Buzzing Confusion. JACQUES MEHLER and ROBIN FOX (eds). Lawrence Erlbaum: 1985. Pp.469. ISBN 0-89859-345-X. Np.

Neural Modulation of Immunity. ROGER GUILLEMIN, MELVIN COHN and THEODORE MELNECHUK (eds). Raven: 1984. Pp.272. ISBN 0-88167-049-9. \$58.50.

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The Pain System: The Neural Basis of Nociceptive Transmission in the Mammalian Nervous System. By WILLIAM D. WILLIS. Karger: 1985. Pp.346. Hbk ISBN 3-8055-3930-4. SwFr. 144, DM 172, \$86.25.

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Phospholipids in the Nervous System, Vol. 2. Physiological Roles. LLOYD A. HORROCKS, JULIAN N. KANFER and GIUSEPPE PORCELLATI (eds). Raven: 1984. Pp.376. ISBN 0-88167-068-5. \$74.

Pocket Atlas of Normal CT Anatomy. By JAMES B. WEINSTEIN, HOSEPH K.T. LEE and STUART S. SAGEL. Raven: 1984. Pp.87. Pbk ISBN 0-88167-070-7. Pbk \$15.

Progress in Biomass Conversion, Vol. 5. DAVID A. TILLMAN and EDWIN C. JAHN (eds). Academic: 1984. Pp.284. ISBN 0-12-535905-5. \$49, £37.

Prostaglandins and Leukotrienes: Blood and Vascular Cell Function. By JONATHAN M. GERRARD. Dekker: 1985. Pp.320. ISBN 0-8247-7259-8. \$65 (US and Canada); £78, SwFr. 195 (elsewhere).

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Secretory Tumours of the Pituitary Gland. PETER McL. BLACK et al. (eds). Raven: 1984. Pp.416. ISBN 0-89004-585-2. \$80.

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Applied Biological Sciences

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Health & Disease: A Reader. NICK BLACK et al. (eds). Open University Press: 1984. Pp.371. Hbk ISBN 0-335-10517-9; Pbk ISBN 0-335-10593-9. Hbk £16; Pbk £5.95.

Health Physics Aspects of the Use of Radioiodines. By DAVID PRIME. Science Reviews Ltd, 28 High Ash Drive, Leeds LS17 8RA: 1985. Pp.55. ISBN 0-905927-76-1. £15.

Hematoporphyrin Derivative Photoradiation Therapy of Cancer. MICHAEL W. BERNIS (ed.). Alan R. Liss: 1984. Pp.134. ISBN 0-8451-0237-0. £23.

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The Medical Renaissance of the Sixteenth Century. A. WEAR et al. (eds). Cambridge University Press: 1984. Pp.349. ISBN 0-521-30112-3. Np.

Microbiological Methods for Environmental Biotechnology. J.M. GRAINGER and J.M. LYNCH (eds). Academic: 1984. Pp.421. ISBN 0-12-295040-2. \$65, £45.

Microspheres and Drug Therapy: Pharmaceutical, Immunological and Medical Aspects. S.S. DAVID et al. (eds). Elsevier: 1984. Pp.448. ISBN 0-444-80577-X. \$109.50, Dfl.285.

Monoamine Oxidase and Disease: Prospects for Therapy with Reversible Inhibitors. K.F. TIPTON, P. DOSTERT and M. STROLIN BENEDETTI (eds). Academic: 1984. Pp.694. ISBN 0-12-691660-8. \$44.50, £34.50.

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Neurotransmitters, Seizures and Epilepsy. RUGGERO G. FARIELLA et al. (eds). Raven: 1984. Pp.391. ISBN 0-88167-057-X. \$68.

Novel Approaches to Cancer Chemotherapy. PRASAD S. SUNKARA (ed.). Academic: 1984. Pp.383. ISBN 0-12-676980-X. \$65, £50.

Nutritional Prevention of Cardiovascular Disease. WALTER LOVENBURG and YUKIO YAMORI (eds). Academic: 1984. Pp.410. ISBN 0-12-456010-5. \$38, £29.50.

Organ Based Autoimmune Diseases. J.M. CRUSE and R.E. LEWIS (eds). Karger: 1985. Pp.278. ISBN 3-8055-3929-0. DM178, \$89.25.

Pharmacology of Intestinal Permeation II. T. Z. CSÁKY (ed.). Springer-Verlag: 1984. Pp.589. ISBN 3-540-13101-9/0-387-13101-9. \$188.20.

Physiological Genetics of Agricultural Crops. By ANDOR BALINT. Akadémiai Kiadó, Budapest: 1984. Pp.167. ISBN 963-05-3288-3. £10.50.

Principles of Health Risk Assessment. By PAOLO F. RICCI. Prentice-Hall: 1985. Pp.417. ISBN 0-13-709197-4. Np.

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Prostacyclin: Clinical Trials. RICHARD J. GRYGLEWSKI, ANDREW SZCZELIK and JOHN C. MCGIFF (eds). Raven: 1984. Pp.159. ISBN 0-88167-051-0. \$41.

Recent Advances in Systemic Lupus Erythematosus. P.H. LAMBERT, L. PERRIN and S. IZUI (eds). Academic: 1984. Pp.359. ISBN 0-12-434620-0. \$35, £24.

Reviews in Perinatal Medicine, Vol. 5. EMILE M. SCARPELLI and ERMELANDO V. COSMI (eds). Alan R. Liss: 1984. Pp.214. ISBN 0-8451-0400-4. £32.

Tissue Culture of Epithelial Cells. MARY TAUB (ed.). Plenum: 1984. Pp.288. ISBN 306-41740-5. Np.

Vasodilator Mechanism, Vol. 38. P.M. VANHOUTE and S.F. VATNER (eds). Karger: 1983. Pp.284. ISBN 3-8055-3903-7. SwFr138, DM165.

ANNOUNCEMENTS

August meeting

21–23 August 1985, **International Workshop on Molecular Biosciences and Bio technology**, Madras, India (Convenors of the workshop, SPIC Main Office, 97 Mount Road, Madras-600 032, India).

September meetings

15–20 September 1985, **International Symposium on Renal Basement Membranes**, London, UK (Dr R. G. Price, Biochemistry Dept., Queen Elizabeth College, Campden Hill Road, London, W8 7AH, UK).

16–20 September 1985, **International Basement Tectonics Conference**, Santa Fe, New Mexico, USA (M. Aldrich, General Chairman, Mail Stop D462, Los Alamos National Laboratory, Los Alamos, New Mexico 87545, USA)

November meeting

21–22 November 1985, **First International Conference on Protein Engineering**, London, UK (Miss H. Raquet, Oyez Scientific and Technical Services Ltd, Bath House, 3rd Floor, 56 Holborn Viaduct, London, UK).

Appointments

Dr Robert Sharpe has been appointed director of the Lord Dowding Fund for Humane Research, in succession to the late Bernard Conyers. The fund actively promotes the development of viable alternative techniques to the use of living animals for research and testing.

Professor John L. Jinks, Vice-Principal of Birmingham University, has been appointed to the board of directors of the Agricultural Genetics Co. Ltd (AGC), the Cambridge-based biotechnology company.

Four new members have joined the Imperial Cancer Research Fund Council. They are **Professor Robert Mahler**, Consultant Physician at Northwick Park Hospital; **Sir John Vane**, Group Research and Development Director, The Wellcome Foundation; **Professor John Evans**, Director of the MRC's Clinical Population Cytogenetics Unit; and **Professor David Weatherall**, Nuffield Professor of Clinical Medicine at the University of Oxford.

Dr J. W. Almond, lecturer in microbiology at the University of Leicester, has been appointed Professor of microbiology at Reading University.

Reading University has appointed **Dr D. L. Pyle** as Professor of biotechnology. He is currently at the Imperial College of Science and Technology, University of London.

The Heriot-Watt University has announced two new professorships. **James L. Murray** has been appointed to a new Chair of Computer-Aided Engineering in the department of mechanical engineering—with effect from 1 May 1985, and **John A. Swaffield** has been appointed to a new Chair of Building Services Engineering in the department of building—from 1 September 1985.

Dr P. G. Hare, at present reader in economics at the University of Stirling, has been appointed Professor of economics at Heriot-Watt University, Edinburgh.

Raymond E. Wetton, Chairman of Polymer Laboratories Ltd, has been appointed Visiting Professor in the department of chemistry at Loughborough University.

Richard K. Root, Professor and vice-chairman of the department of medicine at the University of Washington, has been named the new chairman of the University of California, San Francisco Department of Medicine.

The following have been elected to the Fellowship of Imperial College, London: **Emeritus Professor J.H. Argyris**, Imperial College and University of Stuttgart; **Mr John Egan**, Chairman and Chief Executive, Jaguar Cars Ltd; **The Rt Hon Lord Carr of Hadley**, Chairman, Prudential Corporation and Prudential Assurance Company, Governor, Imperial College; **Mr H.M. Neal**, Chairman, City and Guilds of London Institute; **Professor Sir Randolph Quirk**, Vice-Chancellor, University of London; **Emeritus Professor J. Sutton**, Senior Research Fellow, Centre for Environmental Technology at Imperial College.

Awards

The Simon-Fraser University R. H. Wright award in Olfactory Research has been made to **Professor Karl-Ernst Kaissling** of the University of Seewiesen, FRG. He was cited for his pioneering work in the understanding of the process of olfaction (the sense of smell).

The Royal Society of Chemistry and the Society of Maccabaeans have named the joint winners of this year's Meldola Medal and Prize. **Dr James Darwent**, lecturer in chemistry at Birkbeck College, London, and **Dr Ian Rothwell**, assistant Professor at Purdue University, Indiana, USA, will each receive a bronze medal and £100. The award is made annually to the most promising British chemist under the age of 30.

Dr Mary F. Lyon, FRS, Deputy Director and Head of Genetics Division at the MRC Radiobiology Unit in Oxfordshire has been awarded the 4th Sanremo International Prize for genetic research.

A multidisciplinary team led by **Professor Ronald J. Roberts** at the Institute of Aquaculture, University of Stirling, UK, has been nominated by the British Government for this year's UNESCO Science prize. The nomination recognizes the work of the team on the development of an intensive culture system for farming Tilapia. This is the most important farmed fish of Africa and South-East Asia, which is making an increasing contribution to protein availability in developing countries.

The North Jersey Section of the American Chemical Society has announced that **Alexandra Pines**, Professor of Chemistry at the University of California, Berkeley, has been selected to receive the 1985 Leo Hendrick Baekeland Award. The award, sponsored by Union Carbide Corporation, is made in recognition of his work in the field of magnetic resonance.

Applications are invited for the MRC Senior Fellowships (nonclinical), to arrive before 30 September 1985. The scheme is intended to provide support for a small number of outstanding scientists, and covers personal support for initially the first six years, with provision for supporting staff/expenses/equipment for the first three years. Applicants should be untenured, but with no less than three years' postdoctoral research experience at the date on which they would take up their fellowship. Applicants should be between the ages of 26 and 32 (inclusive) on 30 September 1985, and can obtain further information from the Grants Section at MRC's Headquarters Office (MRC, 20 Park Crescent, London W1N 4AL, UK).

The International Union Against Cancer will award "International Cancer Research Technology Transfer" grants for research on cancer. The purpose of the programme is to promote the transfer of information about new or improved techniques or methods in areas of basic, clinical or behavioural research, in order to further the progress of cancer research. The available funds will permit researchers of any nationality to visit a research centre, or centres, abroad for a period not exceeding 28 days. Information and application forms can be obtained from: International Union against Cancer, Rue du Conseil-General 3, 1205 Geneva, Switzerland.

Miscellaneous

The Voluntary Licensing Authority (VLA) for human *in vitro* fertilization (IVF) has been set up by the Medical Research Council and the Royal College of Obstetricians and Gynaecologists. Centres offering an IVF service or undertaking research in this field may apply to the authority for approval of their work.